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EFFECT OF METALS ON THE DEVELOPMENT OF HYPERACTIVATED MOTILITY BY RABBIT SPERM CELLS

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RESEARCH AND TECHNOLOGY DIRECTORATE

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13. ABSTRACT *(Maximum 200 words)*

Rabbit sperm acquire motion patterns mimicking hyperactivated motility upon incubation in medium M. Lead and cadmium, at concentrations present in seminal plasma of men exposed to the metals, prevented development of these motion patterns in a concentration dependent manner. Nonhyperactivated motility was unaffected. Zinc and mercury, at concentrations that did not inhibit motility, decreased the percentage of sperm with motions that mimicked hyperactivated motility over time, but the decrease was independent of concentration. Concentrations of hexavalent chromium twice that found in seminal plasma of exposed men had no significant effect, as a function of time and concentration, on the percent of sperm with motility mimicking hyperactivated motility. As lead and cadmium are reproductive toxicants, the inhibition of motions that mimic hyperactivated motility in medium M may be useful for the identification of metals and other compounds with the potential to adversely affect reproduction.

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Rabbit	Toxicity tests	Chemical	16. PRICE CODE
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PREFACE

The work described in this report was authorized under Project No. IN6A. This work was started in February 1992 and completed in September 1992.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institute of Health Publication No. 85-23, 1985, as promulgated by the committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council (Washington, DC). These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the Laboratory Animal Use and Review Committee (LAURC), U.S. Army Edgewood Research, Development and Engineering Center (ERDEC), which oversees the use of laboratory animals by reviewing for approval all ERDEC research protocols requiring laboratory animals.

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EFFECT OF METALS ON THE DEVELOPMENT OF HYPERACTIVATED MOTILITY BY RABBIT SPERM CELLS

1. INTRODUCTION

During capacitation, sperm cells develop a vigorous, nonprogressive, random motion characterized by a rapid lateral movement of the head and whiplashing of the tail. This hyperactivated motility is an integral part of capacitation and is required for fertilization.[1,2] Compounds that inhibit hyperactivated motility would also prevent fertilization. Thus, measurement of the inhibition of sperm hyperactivated motility by a compound may have use in in vitro reproductive toxicity assessment. A computer-directed method for the identification and quantitation of hyperactivated rabbit sperm cells or cells possessing motions mimicking hyperactivated motility in a mixed population of hyperactivated and nonhyperactivated cells was recently developed.* This computer model was used to study the effect of lead, cadmium, chromium, mercury, and zinc on the acquisition by rabbit sperm cells of hyperactivated motility or motions that emulated hyperactivated motility, during incubation in a newly formulated medium, where hyperactivated motion patterns develop after incubation for 1 hr.[3] Lead and cadmium were chosen because of their deleterious effect on reproduction, chromium because of its military importance, and zinc and mercury for their apparent benign consequences for reproduction. The results of this study are presented in this report. In this report, hyperactivated motility is used to describe the motions mimicking hyperactivated motility developed by rabbit sperm cells during incubation in medium M[4] even though the physiological status of incubated sperm is unknown.

2. MATERIALS AND METHODS

2.1 Animals.

New Zealand white rabbits were individually housed in standard rabbit cages in a room maintained at 25 ± 3 °C and 50 ± 10% relative humidity (RH) with a 12 hr light/dark cycle. Standard certified laboratory rabbit chow and water were available ad libitum.

*Young, R.J., and Bodt, B.A., Hyperactivated Rabbit Sperm Motility Parameters, MS-1256, U.S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, submitted for publication 20 October 94, UNCLASSIFIED Report.

2.2 Collection and Purification of Sperm Cells.

Sperm cells were collected and purified by centrifugation through a discontinuous Percoll gradient as previously described.[4]

2.3 Videotaping of Sperm Cells.

Videotaping of sperm motion was carried out as previously described.[5-7] A chamber 20 μm deep (2-7 μL/drop) or Microcell slides 20-24 μm depth (10 μL/drop) was used for videotaping. Motility of cells in 2 drops of each sperm cell suspension was recorded on videotape.

2.4 Analysis of Videotapes.

Analysis of videotapes for hyperactivated sperm cells was carried out with the CellTrak system. The following settings were used for the CellTrak system with the VP110 video processor, CellTrak software version 3.15 and CTS/R version 3.21 (Motion Analysis System, Santa Rosa, CA). Frame rate 30 frames/s, duration of capture 30 frames, minimum path length 15 frames, minimum velocity 20 μm/s, maximum velocity 500 μm/s, distance scale factor 1.8393 μm/pixel, camera aspect ratio 1.0, ALH path smoothing factor 7 frames, centroid X search neighborhood 4 pixels, centroid Y search neighborhood 2 pixels, centroid cell size 2 pixels minimum 25 pixels maximum, maximum path interpolation 1 frame, path prediction percentage 0%, sample depth 20 μm. Sperm motility parameters measured by the system were linearity (LIN), curvilinear velocity (VCL), average amplitide lateral head displacement (AALH), and wobble (WOB). Wobble (WOB = VAP/VCL, where VAP is the average path velocity) was obtained by modifying the system Funkey6 batch file with the system EV system. Each of the motility parameters values are the overall averages of the parameters of the cells in seven fields from each of 2 drops of sperm suspension. This procedure was previously determined as optimal for accurate estimation of motility parameters.[7] Only cells that could be tracked for a minimum of 15 frames were used for computation of motility parameter averages.[7] Cells with motility mimicking hyperactivated motility were identified by the threshold values for WOB $\leq$ 0.685 and VCL $\geq$ 55 μm/s, which was previously determined for selection and measurement of hyperactivated sperm.*

*Young, R.J., and Bodt, B.A., Hyperactivated Rabbit Sperm Motility Parameters, MS-1256, U.S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, submitted for publication 20 October 94, UNCLASSIFIED Report.

2.5 Medium.

Incubation was carried out in medium M.[3] The composition of medium M is: KCl 0.03 g, $CaCl_2.2H_2O$ 0.033 g, $MgCl_2.6H_2O$, 0.0106 g, NaCl 0.759 g, Tris.HCl 0.0572 g, Tris 0.0166 g, glucose 0.25 g, and bovine serum albumin (BSA) 300 mg in 100 mL twice glass distilled water. The amount of BSA was reduced to 200 mg/mL for incubations with zinc chloride.

2.6 Sperm Cell Incubation.

Sperm cells, purified by centrifugation through a Percoll gradient, were washed with medium M (5 min, 300xg, room temperature) and then resuspended in medium M. A mixture of washed sperm (5-10 x 10^6 cells), the chloride salt of lead, cadmium, zinc, mercury, or potassium dichromate, in medium M (1 mL final) contained in a 100 x 13 mm culture tube, was incubated in air at 37 °C. A tube containing sperm cells only, suspended in medium M, served as the control. At 0 time, cell motility in 2 drops of the control cell suspension was recorded on videotape. This was repeated at intervals of 0.5, 1, 2, and 4 hr after commencement of incubation with the control and with the sperm suspensions exposed to the metal salts. Sperm cells were exposed to three concentrations of each salt, and each treatment was repeated 3 to 5 times. The salts used, their concentration ranges, and the number of replicates were: lead chloride, 25-5 μM, 5 replicates; zinc chloride, 200-50 μM, 3 replicates; mercuric chloride, 1-0.2 μM, 4 replicates; cadmium chloride, 100-20 μM, 3 replicates; and potassium dichromate, 5-1 μM, 3 replicates. Sperm were obtained from different rabbits for each replicate except for experiments with lead when sperm were obtained from four and not five rabbits.

2.7 Statistical Analysis.

Rabbits (sperm cells) were considered as random blocks, and time and concentration were considered as fixed effects in a 4 x 4 factorial (4 concentrations x 4 times) design within a randomized block design.[8]

All data analyses were accomplished using the statistical software SYSTAT, version 5, 1992 (SYSTAT, Inc., Evanston, IL) Multivariate analysis of variance (MANOVA) was performed, testing for the equivalence of response vectors over the experimental conditions considered. Only time, concentration, and their interaction were testable. Each test was performed at the α=0.05 level. If significant effects were observed, they were further examined using univariate analysis of variance (AOV), supported by pair-wise comparisons and numerous graphical procedures including parallel boxplots, scatterplot matrices, three dimensional scatterplots, and localized data smoothing procedures. The smoothing procedure distance weighted

least squares (DWLS) was used to smooth three dimensional scatterplots, and LOWESS was used to suggest the trend in the scatterplot matrices. In addition, so points in the scatterplot matrices would not be obscured by close neighboring points, they were plotted in a "jittered" fashion, adding small random noise to the (x,y) coordinate for the point. The DWLS, LOWESS, and jittering are described in SYSTAT. The usual transformation, arcsine of the square root of the sample proportion, was made to the percent of motile sperm that were hyperactivated (PMOTHY) and the percent of motile sperm (PMOT) to better satisfy the assumptions of the AOV. (All graphs appear in original units.) When significant effects were not observed at the α=0.05 level, but graphical analysis suggested *consistent* structure in the data, the p-values were reported to add to the graphical summary.

Although six motility characteristics were measured, separate analyses were conducted, one using PMOTHY and PMOT as the response vector and the other using AALH, LIN, VCL, and WOB for each of the five metals under study. The reasons for this approach are

- The PMOTHY and PMOT are more important and are naturally linked in the interpretation of results, how they are linked will be discussed.

- Where there were data points for each motility parameter, the correlation between the transformed values of PMOTHY and PMOT and any of the other four motion parameters was not strong enough to raise concern in analyzing the response vectors separately.

3. RESULTS

3.1 Motility Inhibition.

The three concentrations of each salt employed in the study are shown in the Table. The concentrations of lead and cadmium chlorides, used in the study, spanned the concentration range of the metals found in seminal plasma of men exposed to the metals.[9,10] The highest concentration used was the one that did not inhibit motility. In the case of potassium dichromate, the highest concentration used was about twice that of Cr^{+6}, which was found in seminal plasma of workers exposed to the metal.[11] Sperm cell motility was not visibly inhibited by the salt at a concentration that was 2 to 5 times this over a 2-4 hr inhibition period. Zinc chloride formed a precipitate when added to medium M at the concentration reported for seminal plasma zinc,[12,13] and sperm cells were not motile. Sperm remained motile for 4 hr when the amount of BSA was reduced to 2 mg/mL and the zinc chloride concentration was reduced to 200 μM (which was lower than that of zinc in semen). One to 2 μM $HgCl_2$ was the highest concentration

Table. Concentration of Compounds

	Concentration			
Compound	High	Medium	Low	Replicates*
$CdCl_2$	100 μM	50 μM	20 μM	3
$PbCl_2$	25 μM	10 μM	5 μM	5
$HgCl_2$	1 μM	0.5 μM	0.2 μM	4
$ZnCl_2$	200 μM	100 μM	50 μM	3
K_2CrO_7	5 μM	2.5 μM	1 μM	3

*Replicates, except for $PbCl_2$, were carried out with sperm cells from different rabbits. For $PbCl_2$ sperm were obtained from four rabbits.

of the salt in which sperm remained motile for 4-6 hr. Clearly, rabbit sperm cell motility was most sensitive to mercuric ions, concentrations of mercuric chloride > 1-2 μM inhibiting motility. The consequences for sperm motility and the development of hyperactivated motility upon exposure of cells salt concentrations that did not inhibit motility were complex and are reported for each salt.

3.2 Mercury.

The mercury data set for PMOTHY and PMOT consisted of 64 observations, 4 rabbits X 4 concentrations X 4 times. MANOVA showed no significant interaction, as determined by the Wilks Lambda test statistic, between time and concentration, and no significant concentration effect; however, a difference in the response vector over time was observed. The AOV indicated that PMOTHY and PMOT were affected by time. The mean percents recorded for the response vector (PMOTHY and PMOT) were 29.1, 66.5; 26.8, 62.9; 16.0, 64.1; and 9.8, 55.7, respectively, for 0.5, 1, 2, and 4 hr. Tukey's pair-wise comparisons showed significant differences in PMOTHY to occur between 0.5 hr, 2 and 4 hr, and between 1 and 4 hr. For PMOT, a difference was observed only between 0.5 and 4 hr. The negative association observed between PMOTHY and time was consistent among rabbits as indicated by smoothed scatterplots; whereas, it appeared that the negative association found between PMOT and time was principally attributable to 2 of the 4 rabbits (Figure 1a-e, Figure 2a and b).

 Text continues on page 21.

a. All Rabbits (Hg)

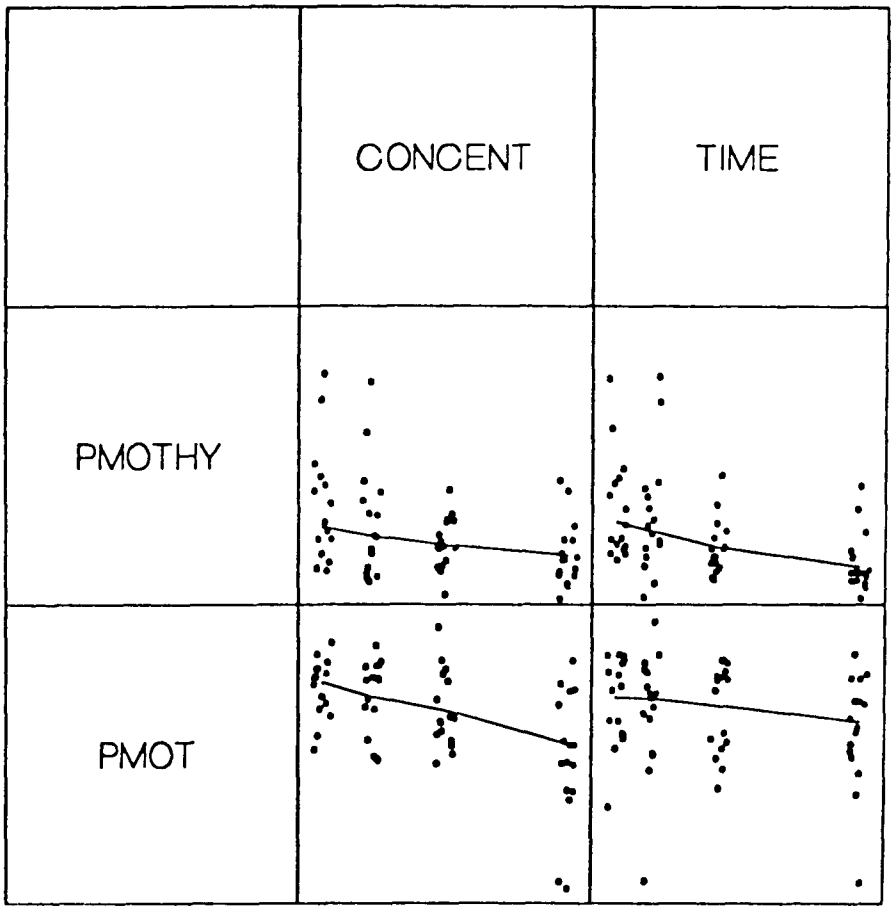


Figure 1. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 0.2 μM, 0.5 μM, and 1.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr)

b. Rabbit 38 (Hg)

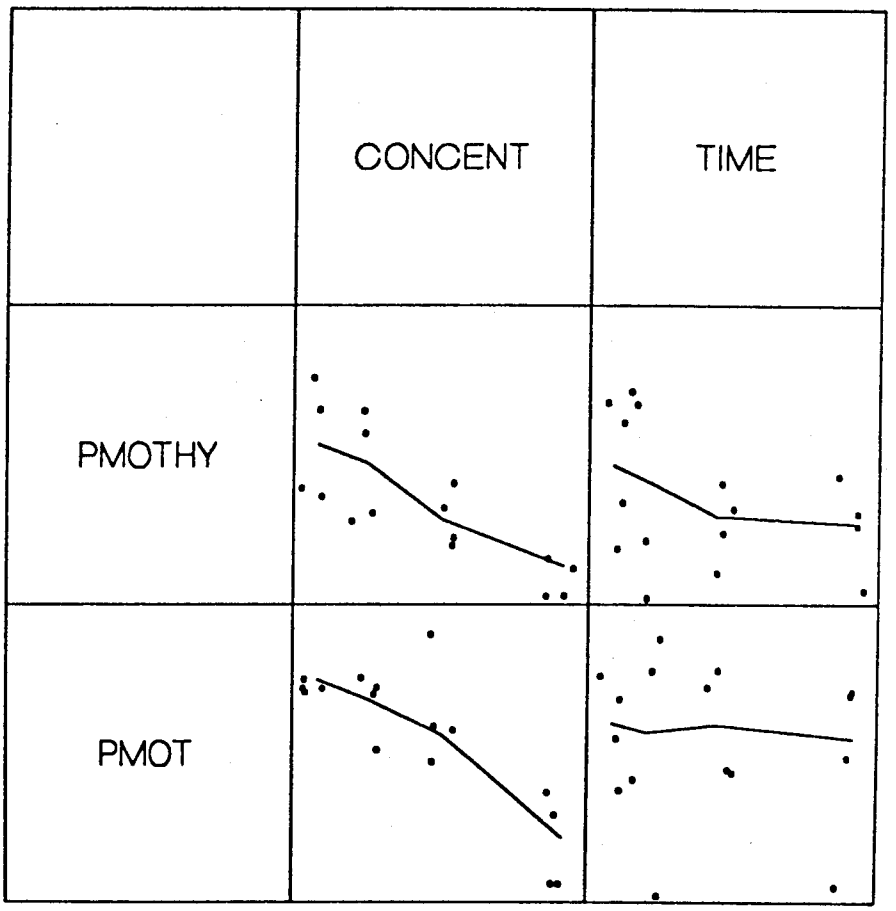
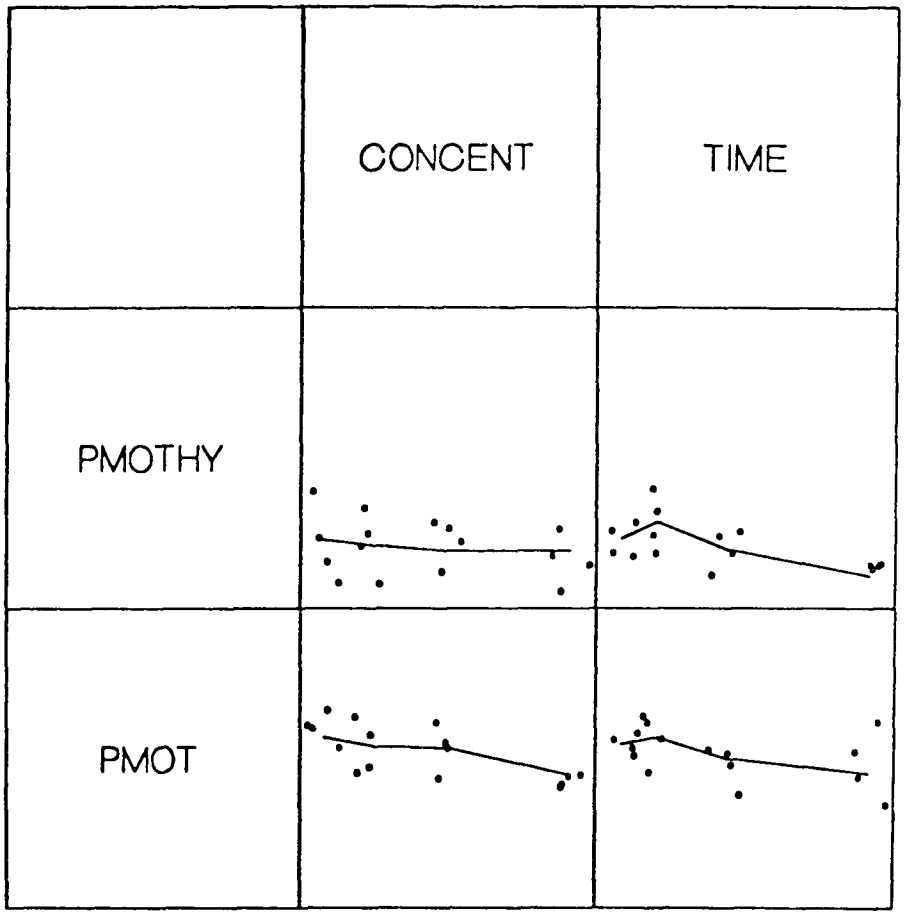


Figure 1. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 0.2 μM, 0.5 μM, and 1.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

c. Rabbit 712 (Hg)



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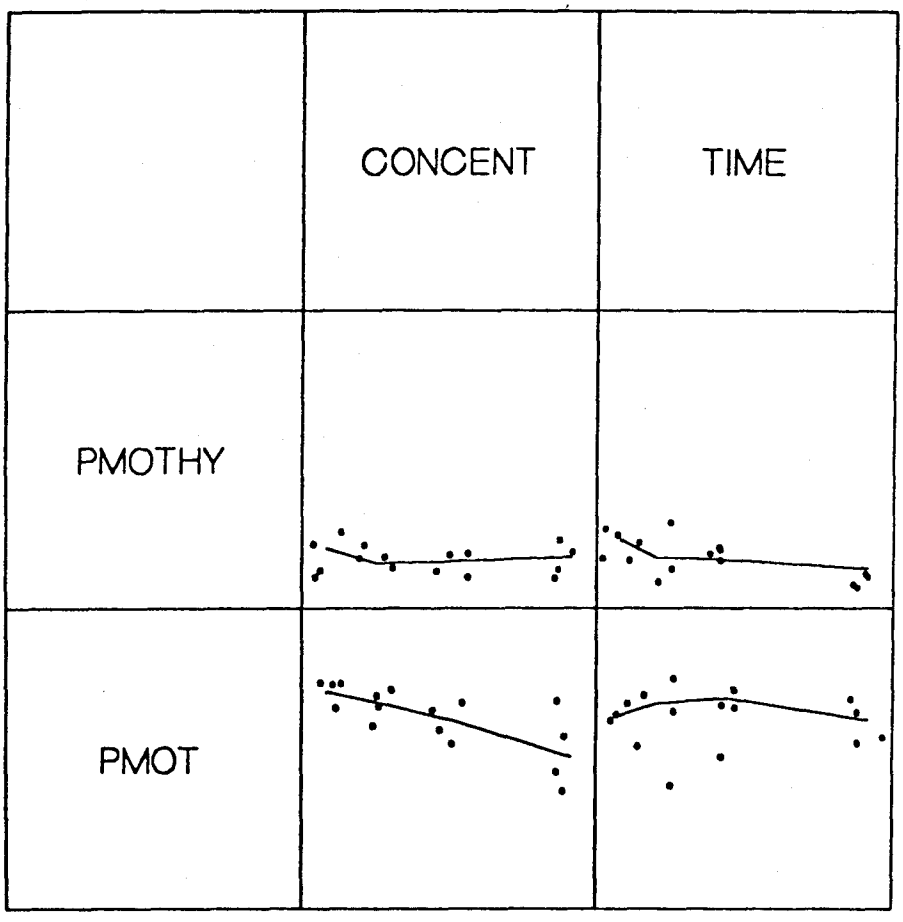
d. Rabbit 864 (Hg)



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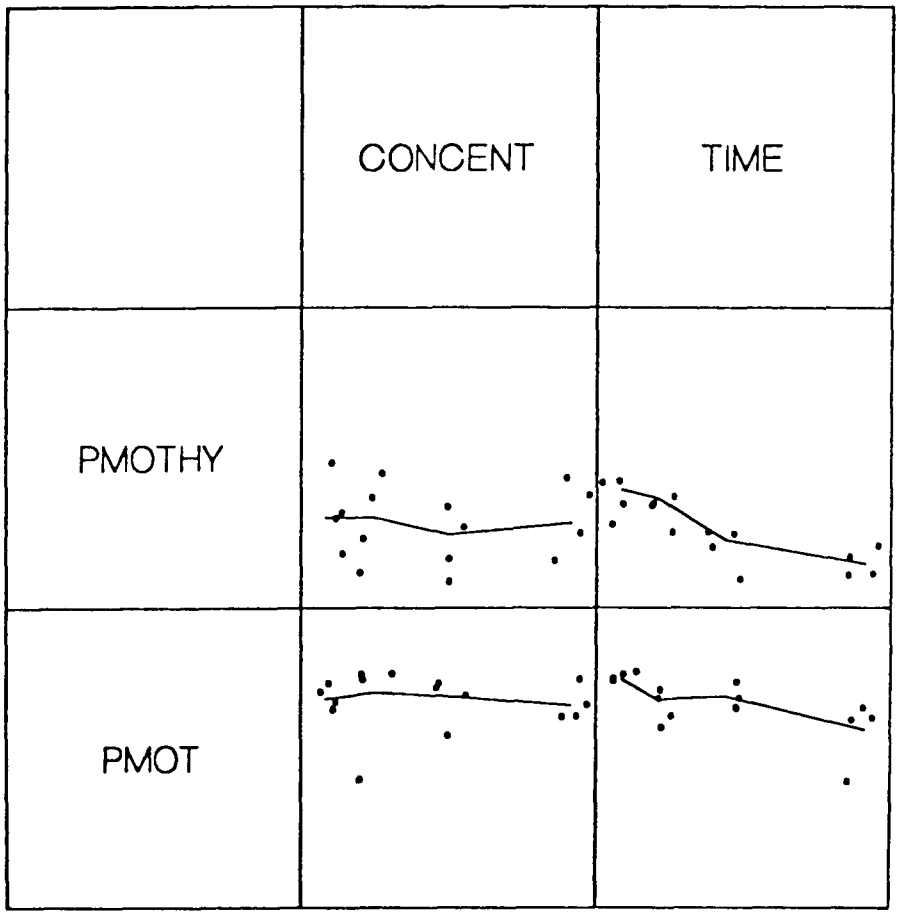
e. Rabbit 967 (Hg)



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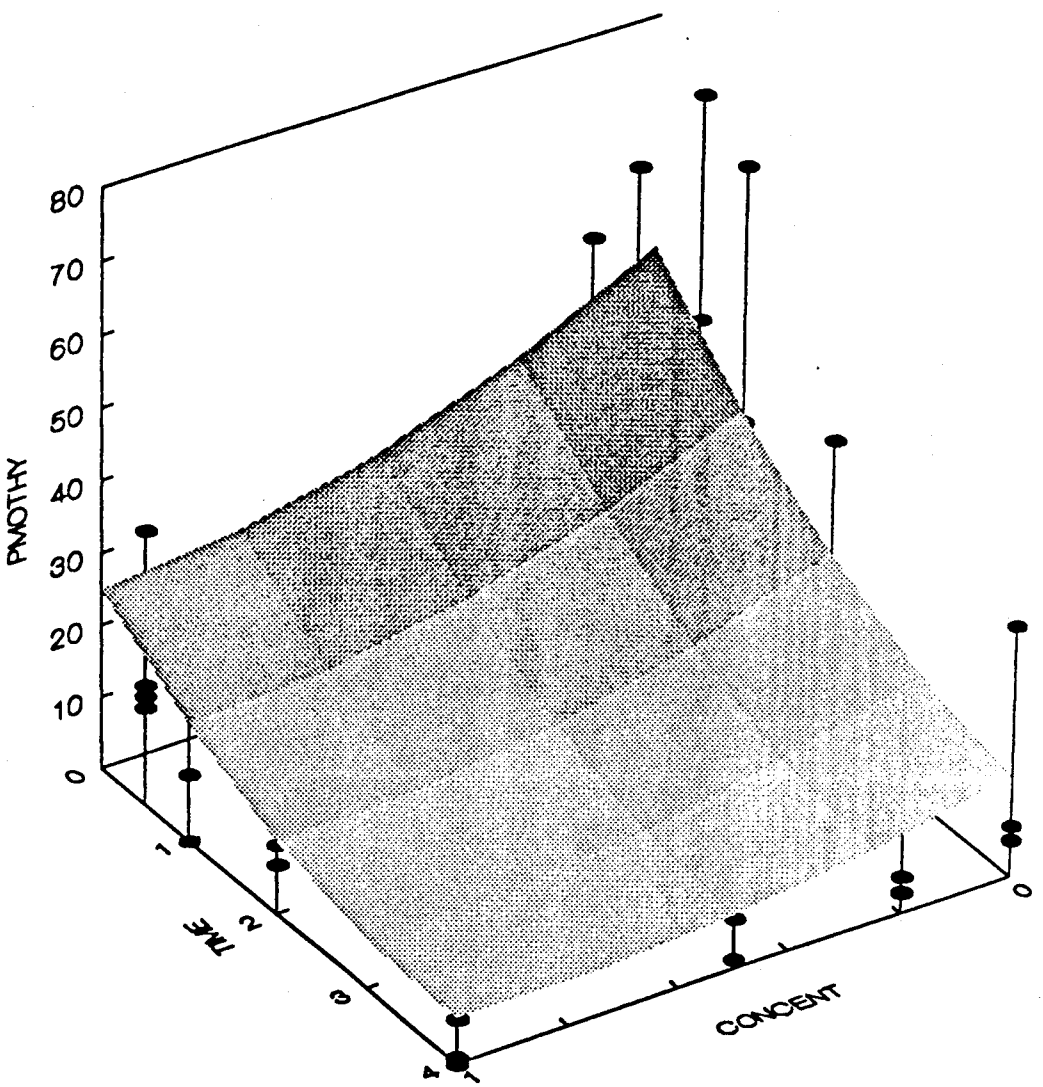


Figure 2. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Hg Concentration (μM) and Time (hr)

b. PMOT (Hg)

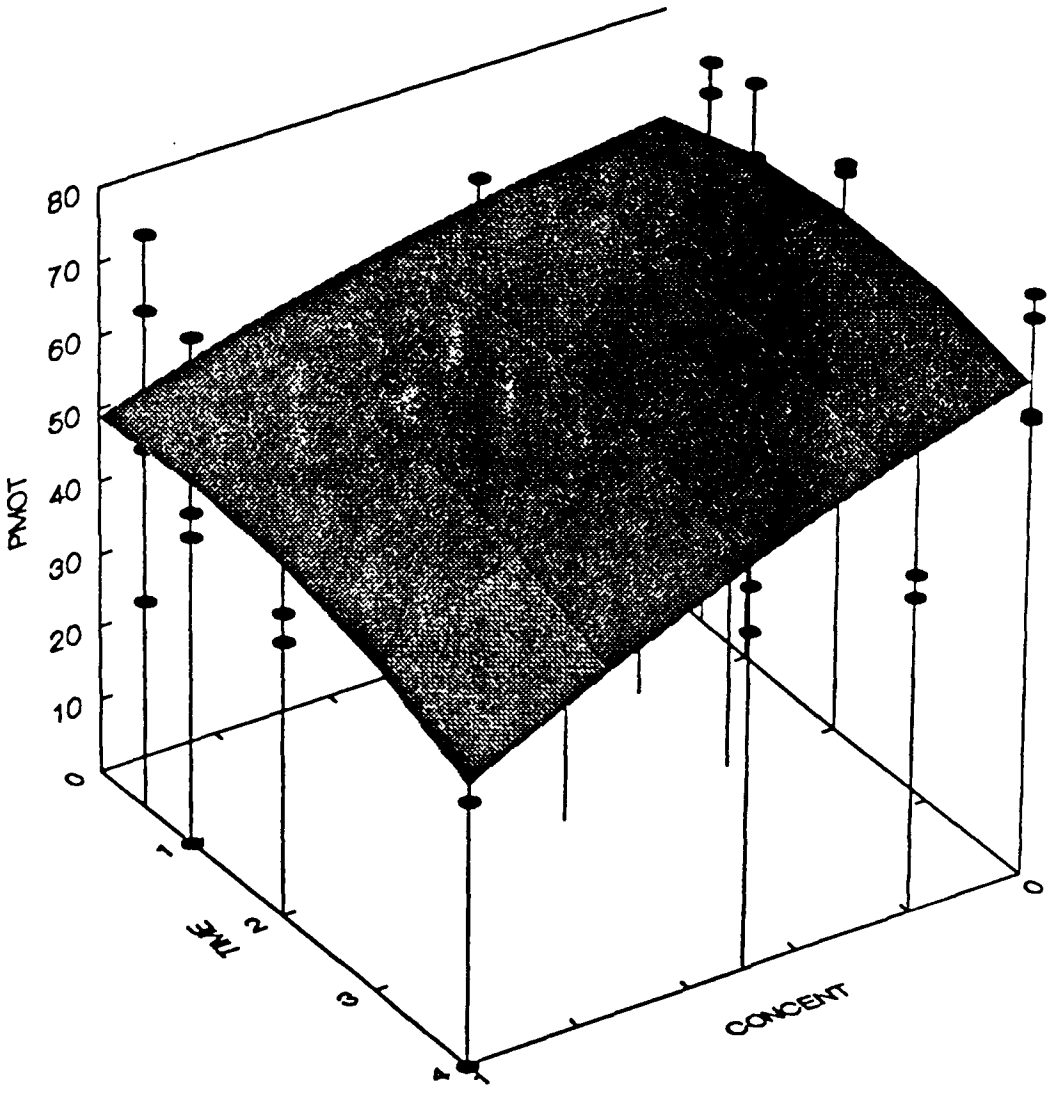


Figure 2. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Hg Concentration (μM) and Time (hr) (Continued)

Figure 1a also shows a trend due to decreasing PMOT over increasing concentration. This trend was not significant but did contribute to a p-value of 0.098 in the MANOVA test of the concentration effect on the response vector. The visually apparent trend is greatly influence by two near zero values from rabbit 38 (Figure 1b).

Nonhyperactivated motility, represented by the response vector (AALH, LIN, VCL, WOB), was not found by the MANOVA to be affected by concentration or time. In Figure 3, values for each of the four motility parameters appear to decrease slightly with the highest concentration, while drifting up initially over time. However, the MANOVA p-values for concentration and time effects on the response vector were 0.084 and 0.138, respectively, indicating that the changes were not statistically significant.

3.3 Zinc.

The zinc data set for PMOTHY and PMOT consisted of 48 observations, 3 rabbits X 4 concentrations X 4 times. MANOVA showed no significant interaction between time and concentration but did show a difference in the response vector over time and concentration, individually. The AOV indicated that the differences were due to PMOTHY over time and to PMOT over concentration. Over times 0.5, 1, 2, and 4 hr, the response vector mean values for PMOTHY, PMOT, were 31.3, 68.6; 31.4, 66.8; 17.0, 64.2; and 9.3, 54.5, respectively. Pair-wise comparisons suggested that the differences in PMOTHY over time occurred between 0.5 and 1 hr as compared with 2 and 4 hr. Scatterplots suggest that the negative association between PMOTHY and time was consistent among rabbits (Figure 4a-d). Over the concentrations, control, low, medium, and high, the response vector values were 24.4, 69.4; 20.2, 66.1; 21.7, 65.5; and 22.7, 53.3, respectively. Pair-wise comparisons show that the difference in PMOT occurred between the highest concentration and each of the others. Graphically, this negative association appeared consistent among rabbits; however, the statistical significance of the result hinged on one point. A zero for PMOT was observed for one sample when exposed at the highest concentration for 4 hr (Figure 4d). Although this point was statistically an outlier, there was no known physical justification for removing it from the analysis. Graphs depicting the combined influence of time and concentration on the response vector appear as Figure 5a and b.

There were no significant differences noted by MANOVA for the response vector (AALH, LIN, VCL, and WOB) as influenced by concentration or time. In Figure 6, all parameters except AALH appear to drop in value at the highest concentration, but this difference has only a p-value for the MANOVA of 0.461.

 Text continues on page 30.

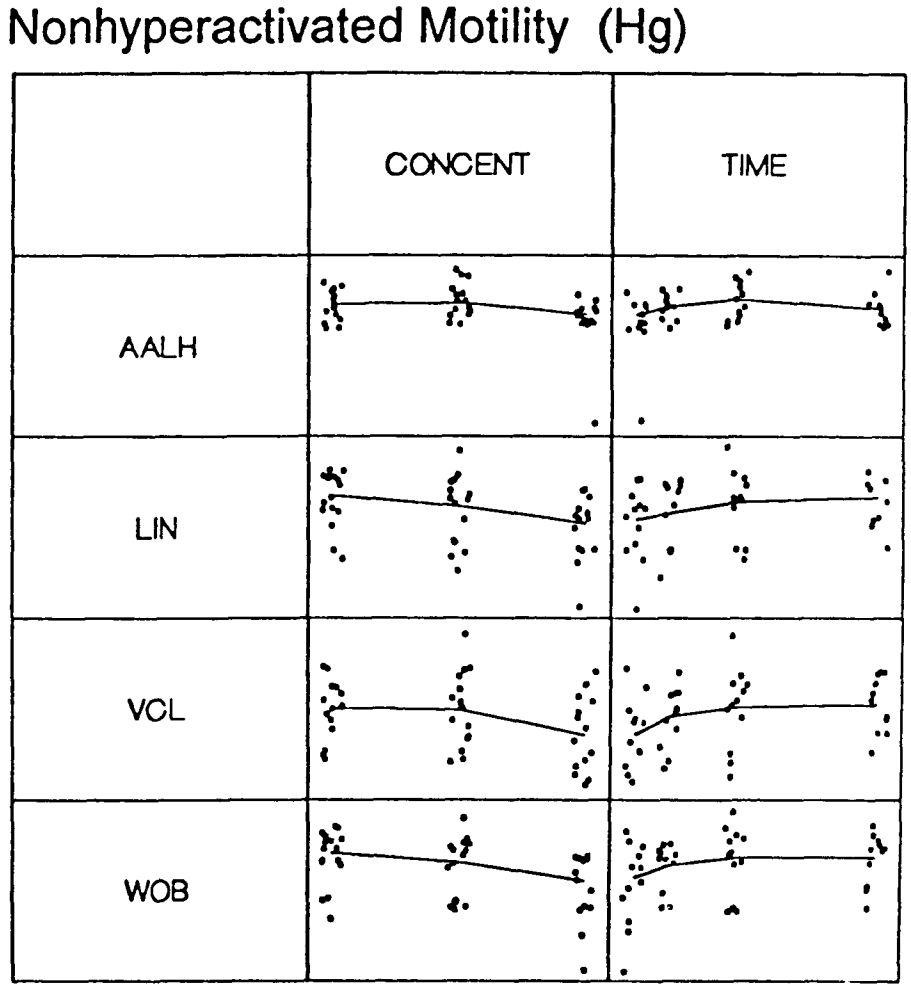


Figure 3. Scatterplot Matrix Depicting the Association Between Each of Hg Concentration (x-Axis Left to Right: 0 μM, 0.5 μM, and 1.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) and Nonhyperactivated Motility Represented by the Motility Parameters: AALH (Minimum 0.4 μm, Maximum 5.7 μm), LIN (Minimum 0.17, Maximum 0.88), VCL (Minimum 36 μm/s, Maximum 115 μm/s), and WOB (Minimum 0.30, Maximum 0.92)

a. All Rabbits (Zn)

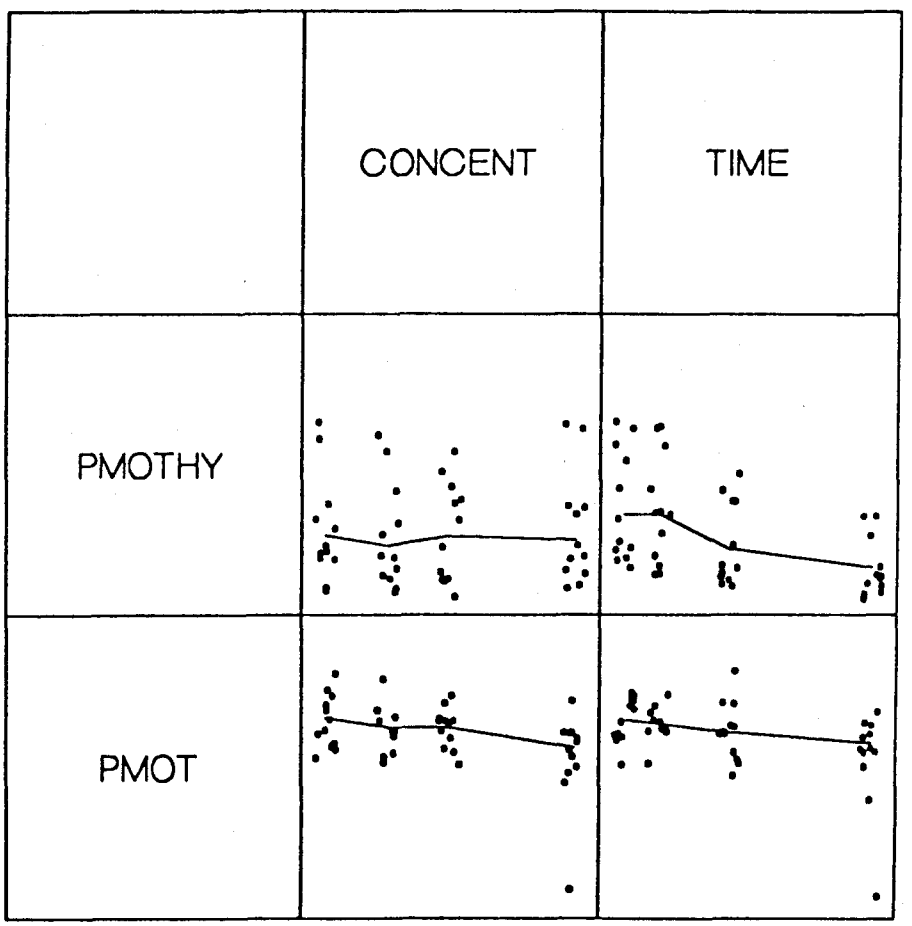


Figure 4. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 50 μM, 100 μM, and 200 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr)

b. Rabbit 38 (Zn)

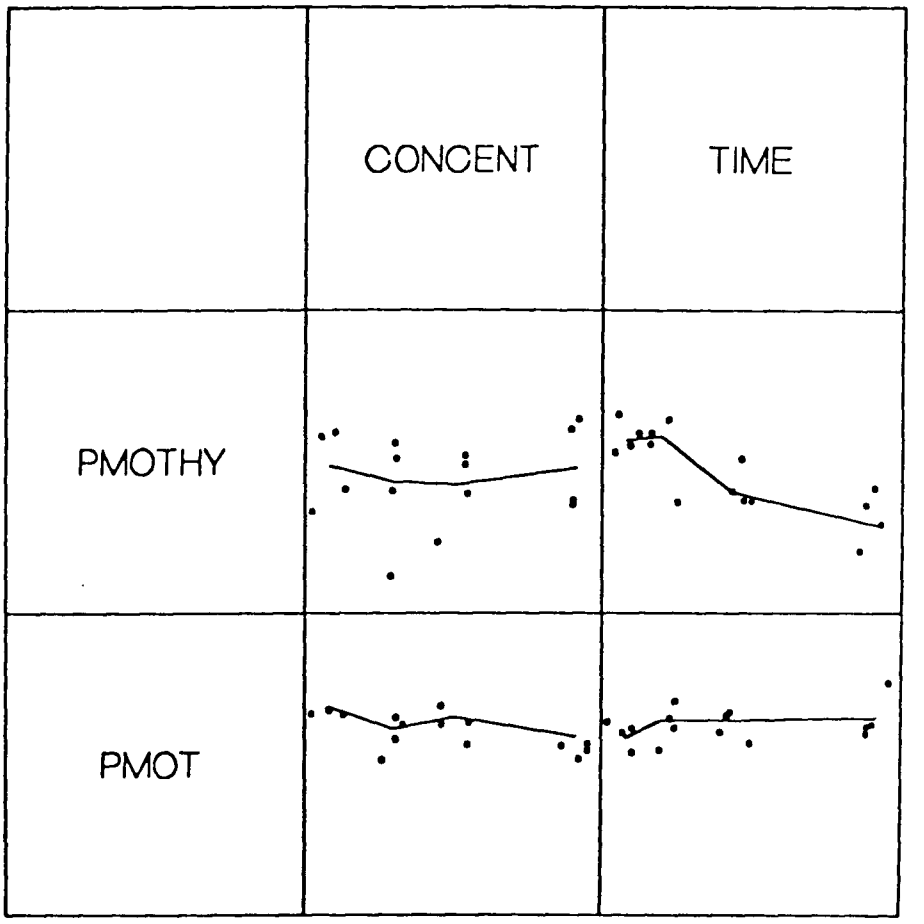


Figure 4. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 50 μM, 100 μM, and 200 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

c. Rabbit 712 (Zn)

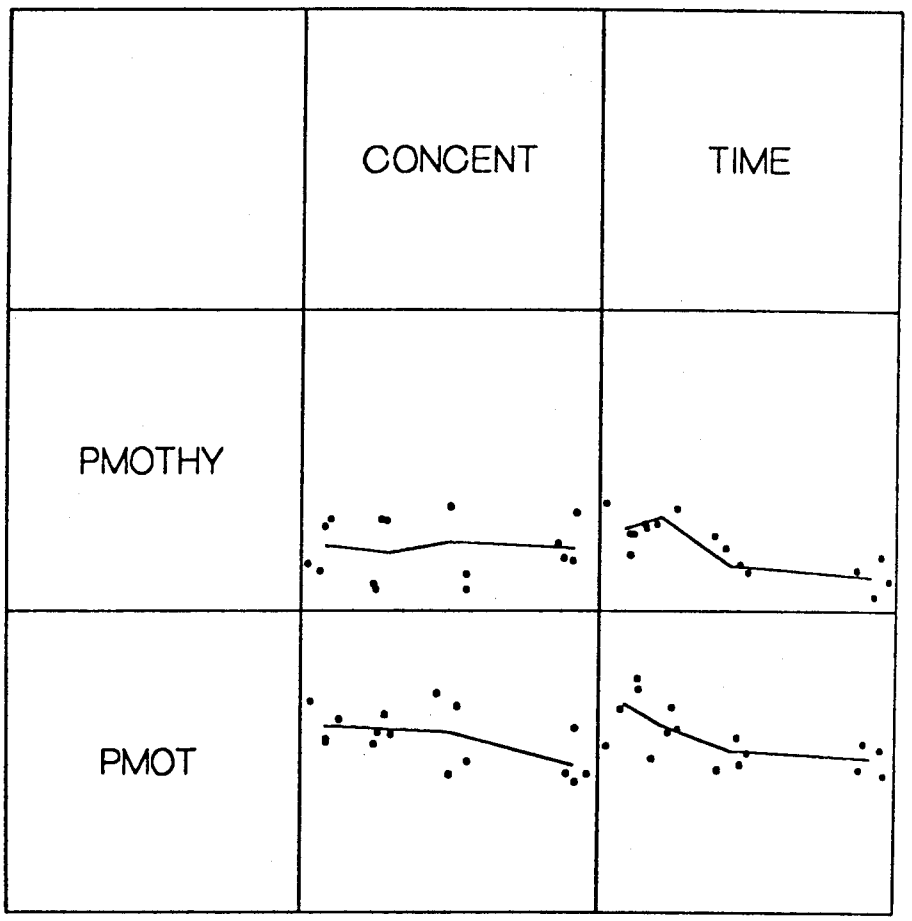
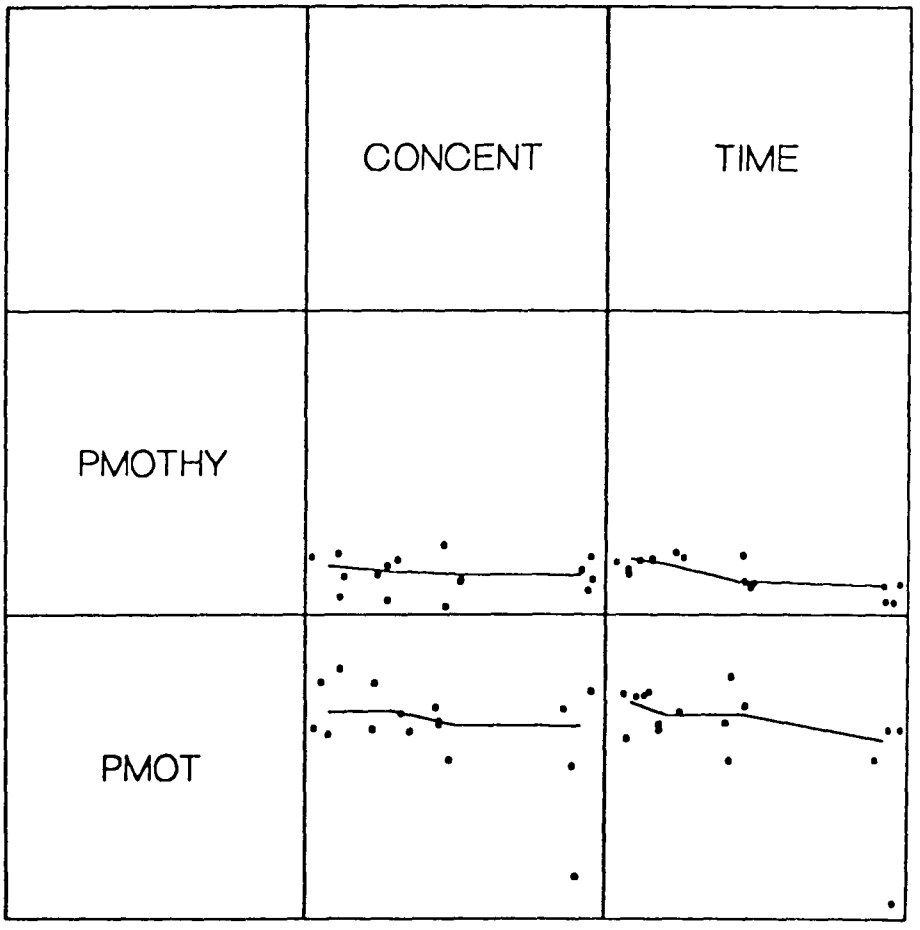


Figure 4. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 50 μM, 100 μM, and 200 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

d. Rabbit 793 (Zn)



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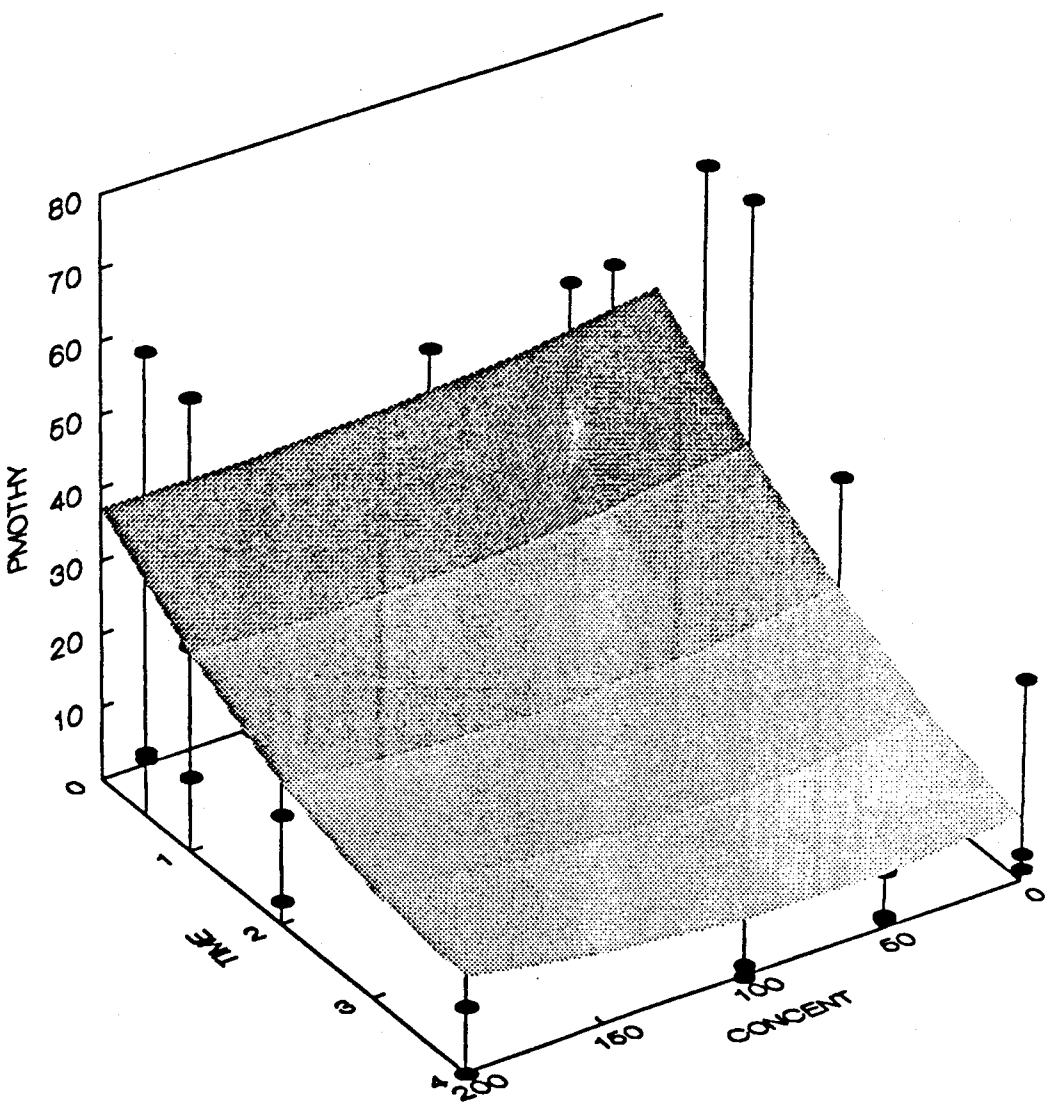

Figure 5. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Zn Concentration (μM) and Time (hr)

b. PMOT (Zn)

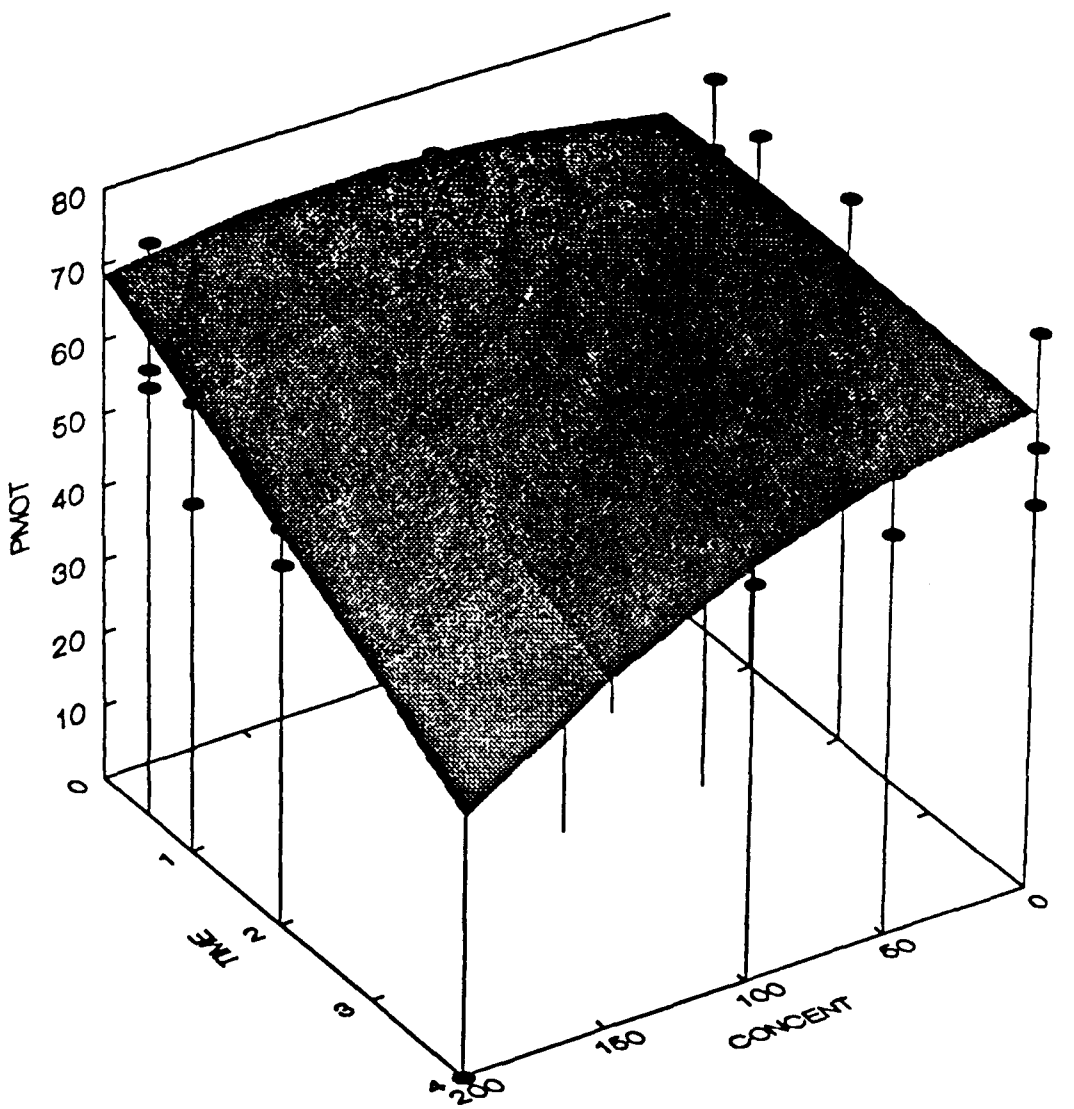

Figure 5. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Zn Concentration (μM) and Time (hr) (Continued)

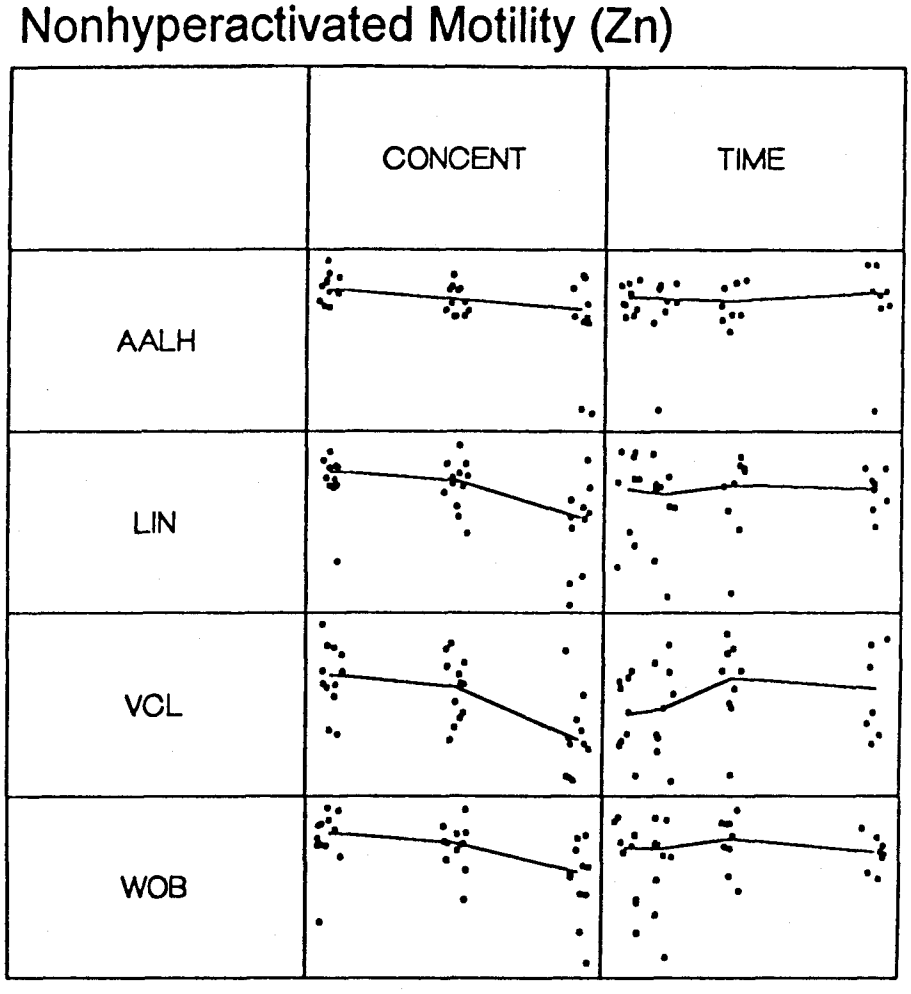

Figure 6. Scatterplot Matrix Depicting the Association Between Each of Zn Concentration (x-Axis Left to Right: 0 μM, 100 μM, and 200 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) and Nonhyperactivated Motility Represented by the Motility Parameters: AALH (Minimum 0.3 μm, Maximum 5.2 μm), LIN (Minimum 0.29, Maximum 0.86), VCL (Minimum 41 μm/s, Maximum 94 μm/s), and WOB (Minimum 0.42, Maximum 0.91)

3.4 Cadmium.

The cadmium data set for PMOTHY and PMOT consisted of 48 observations, 3 rabbits X 4 concentrations X 4 times. MANOVA showed no significant interaction between time and concentration, and no significant time effect; however, a difference in the response vector over concentration was observed. The AOV indicated that the difference was due to PMOTHY. Over concentrations control, low, medium, and high, the response vector values for PMOTHY, PMOT were 25.3, 78.9; 25.3, 72.1; 15.1, 72.0; and 12.2, 63.0, respectively. Pair-wise comparisons suggested that the differences in PMOTHY across concentrations occurred between the control and low concentrations compared with the highest concentration. The negative association between PMOTHY and concentration was consistent among rabbits (Figure 7a-d).

Figure 7a also depicts a drop in both PMOTHY and PMOT over time. This drop was not significant, but the p-value for the MANOVA test was 0.111. This is again seen, along with the concentration influence, in Figure 8a and b.

There was a significant interaction indicated by MANOVA between time and concentration with respect to the response vector (AALH, LIN, VCL, and WOB). The AOV indicated that the only significant difference was attributable to VCL. For the control, the mean VCL was recorded as 55.3, 82.6, 92.5, and 88.2, respectively, for times 0.5, 1, 2, and 4 hr. For the medium and high concentrations, this sequence of VCL over time was 70.4, 82.0, 79.7, and 56.8; and 58.4, 74.6, 64.2, and 50.5, respectively. The difference detected between the control and the medium and high concentrations is that the former values of VCL rise from 0.5 to 1 hr and then remain relatively high; whereas, the latter two each show an initial rise in VCL followed by a drop, particularly by 4 hr. This interaction was consistent among rabbits.

Figure 9 shows each of the responses AALH, LIN, VC, and WOB over concentration and time.

3.5 Chromium.

The chromium data set for PMOTHY and PMOT consisted of 48 observations, 3 rabbits X 4 concentrations X 4 times. MANOVA showed no significant influence on the response vector caused by time, concentration, or their interaction (Figure 10a-d).

Graphically, there is a consistent, over rabbits, negative association between PMOTHY and concentration and PMOTHY and time (Figure 10a-d, Figure 11a and b). MANOVA, acting on both responses simultaneously, found neither pattern to be significant. The p-values for concentration and time were 0.162 and 0.225, respectively.

 Text continues on page 44.

a. All Rabbits (Cd)

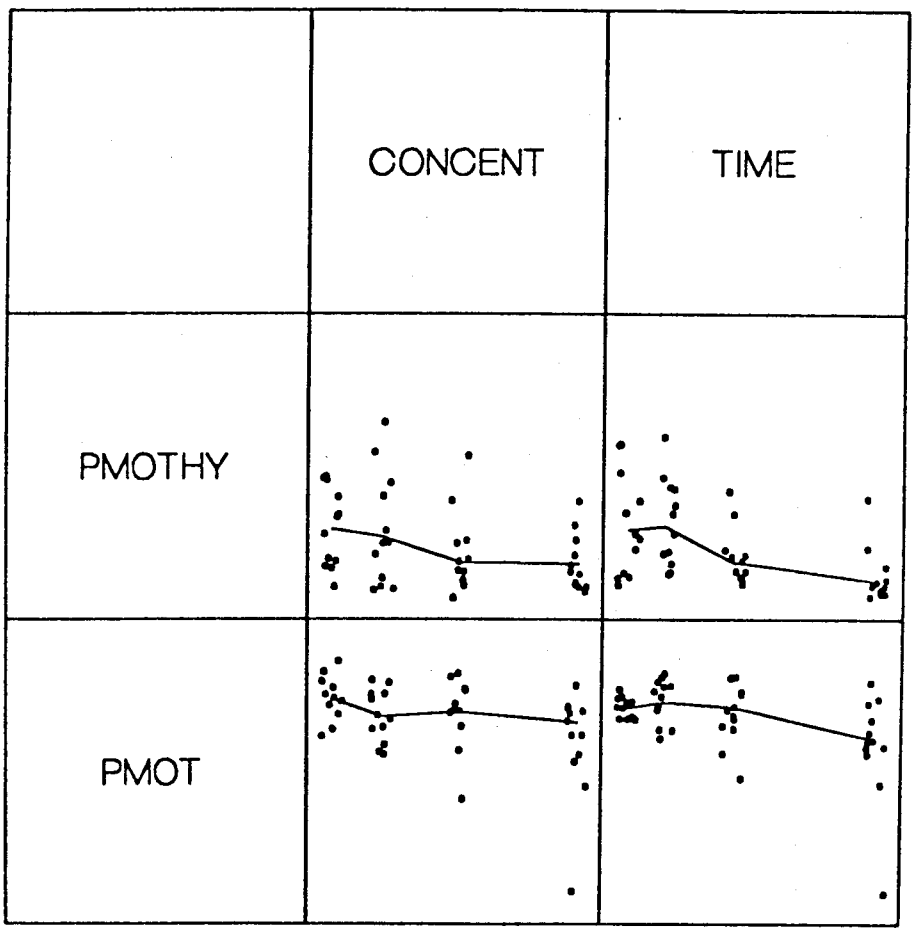

Figure 7. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 20 μM, 50 μM, and 100 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr)

b. Rabbit 38 (Cd)

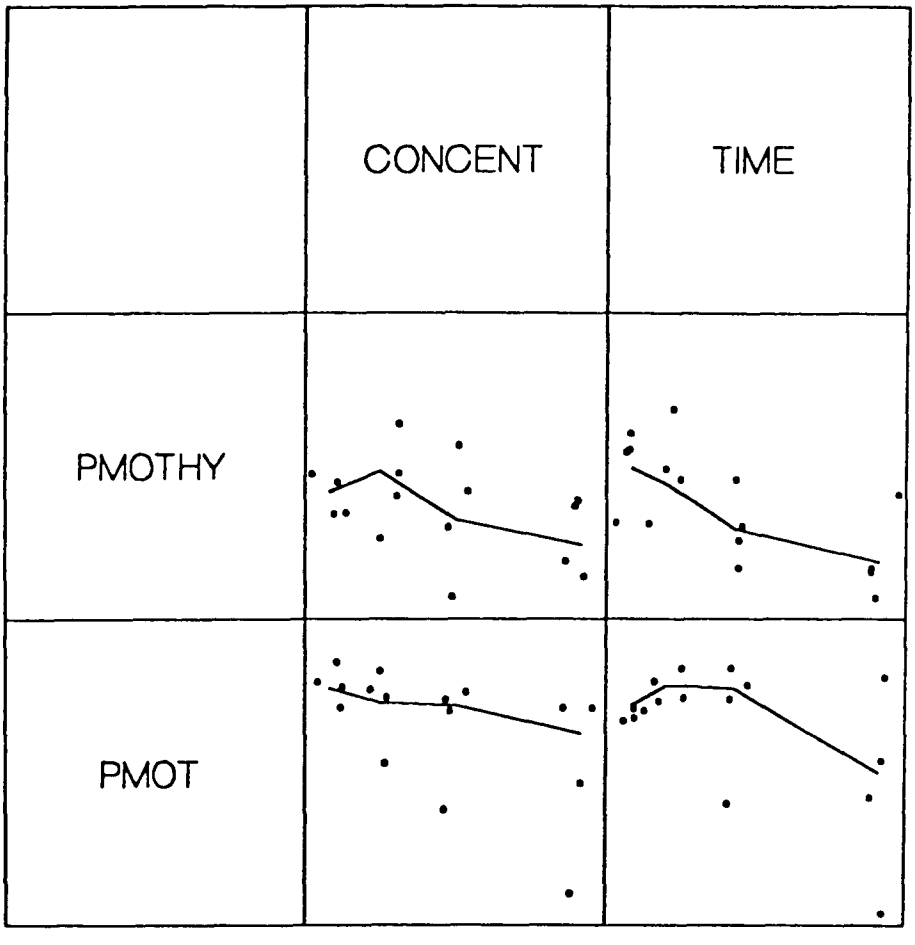

Figure 7. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 20 μM, 50 μM, and 100 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

c. Rabbit 213 (Cd)

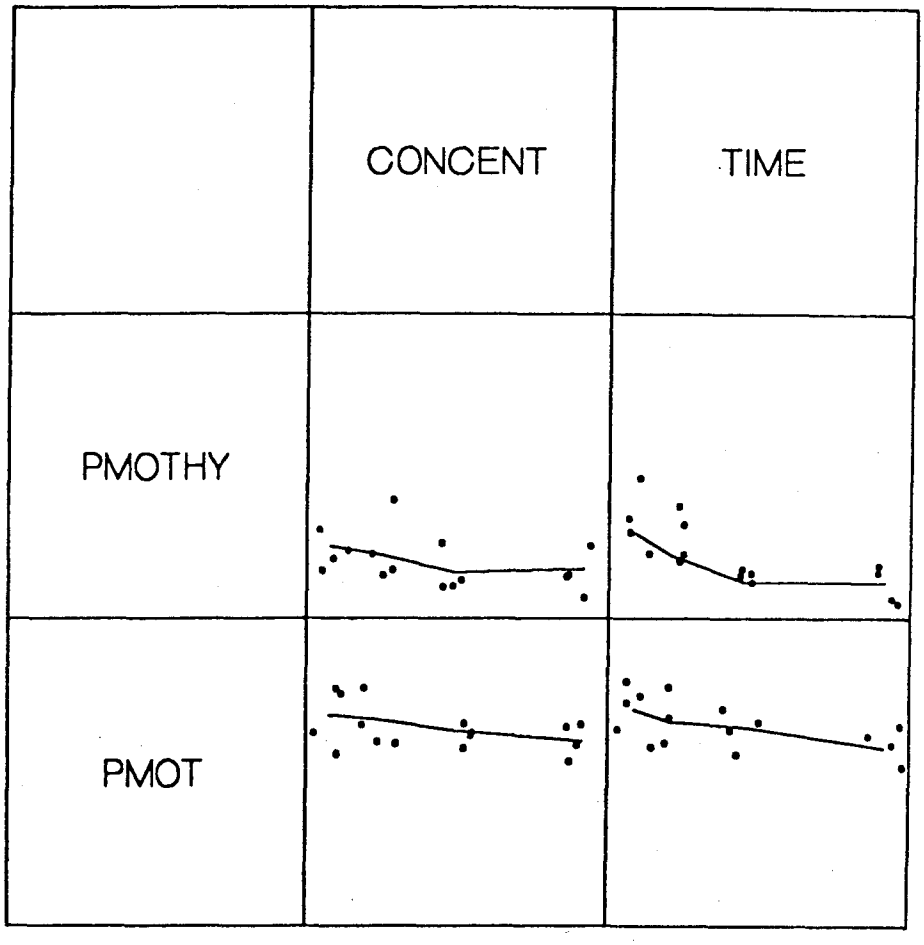

Figure 7. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 20 μM, 50 μM, and 100 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

d. Rabbit 793 (Cd)

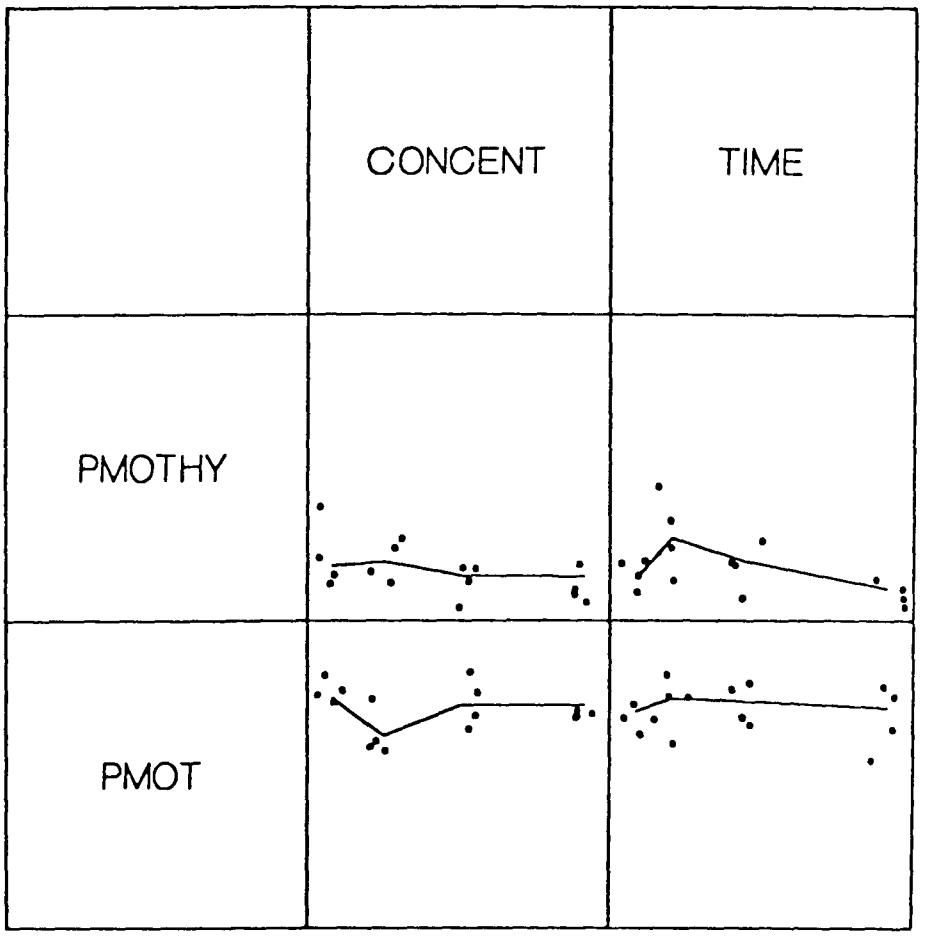

Figure 7. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 20 μM, 50 μM, and 100 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

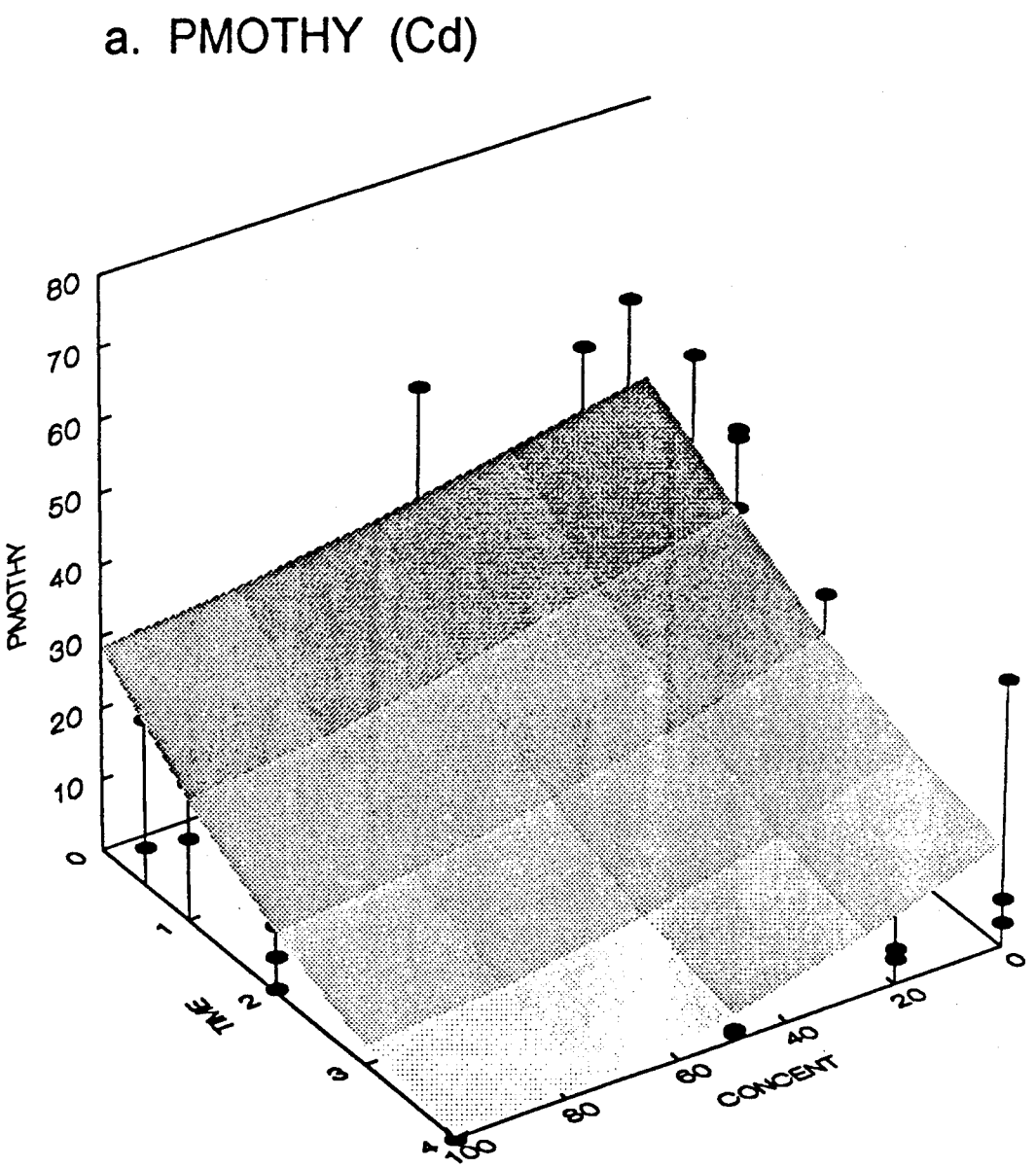

Figure 8. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Cd Concentration (μM) and Time (hr)

b. PMOT (Cd)

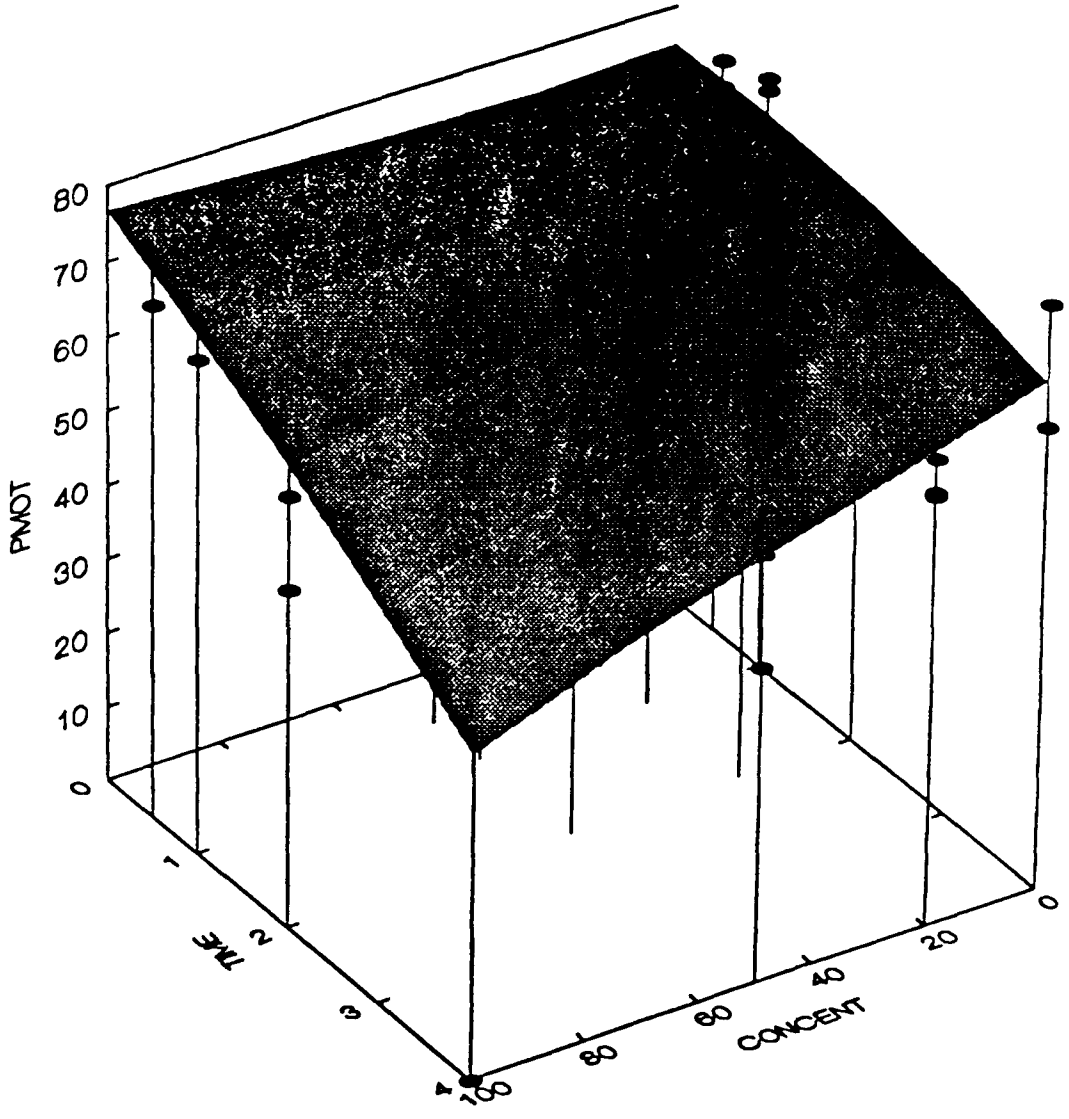

Figure 8. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Cd Concentration (μM) and Time (hr) (Continued)

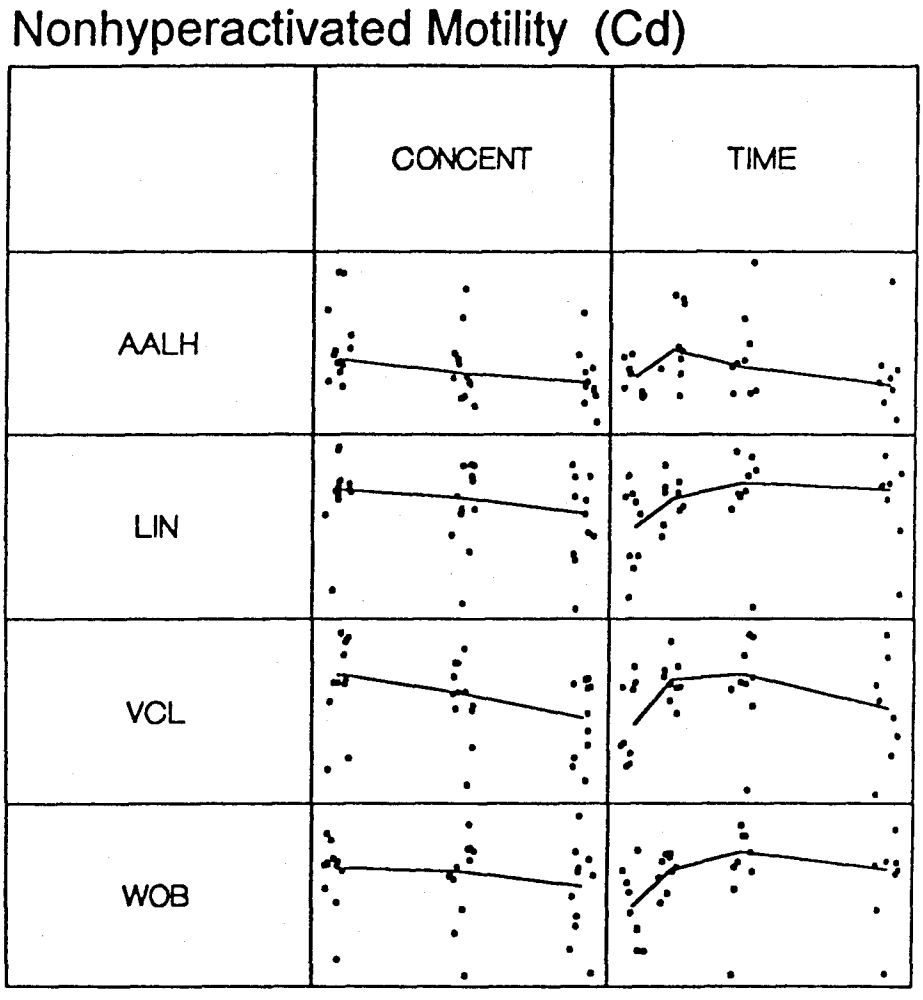

Figure 9. Scatterplot Matrix Depicting the Association Between Each of Cd Concentration (x-Axis Left to Right: 0 μM, 50 μM, and 100 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) and Nonhyperactivated Motility Represented by the Motility Parameters: AALH (Minimum 3.1 μm, Maximum 6.5 μm), LIN (Minimum 0.31, Maximum 0.87), VCL (Minimum 34 μm/s, Maximum 99 μm/s), and WOB (Minimum 0.45, Maximum 0.96)

a. All Rabbits (Cr)

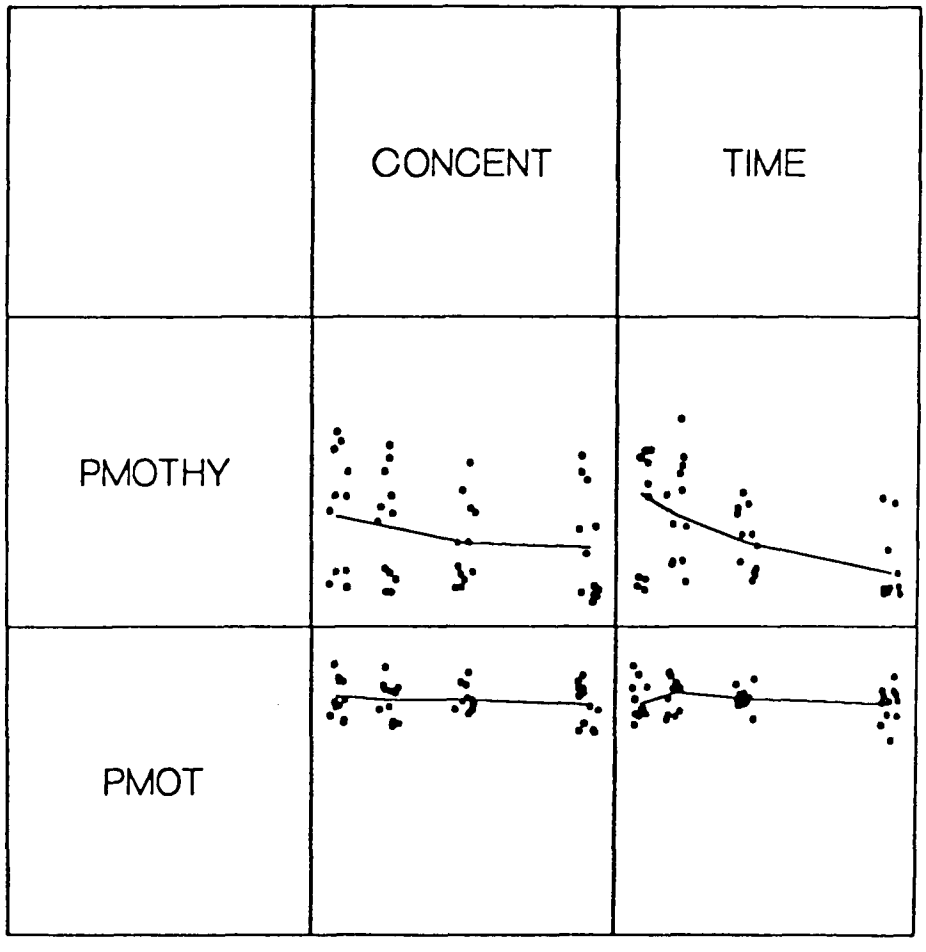

Figure 10. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 1.0 μM, 2.5 μM, and 5.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr)

b. Rabbit 38 (Cr)

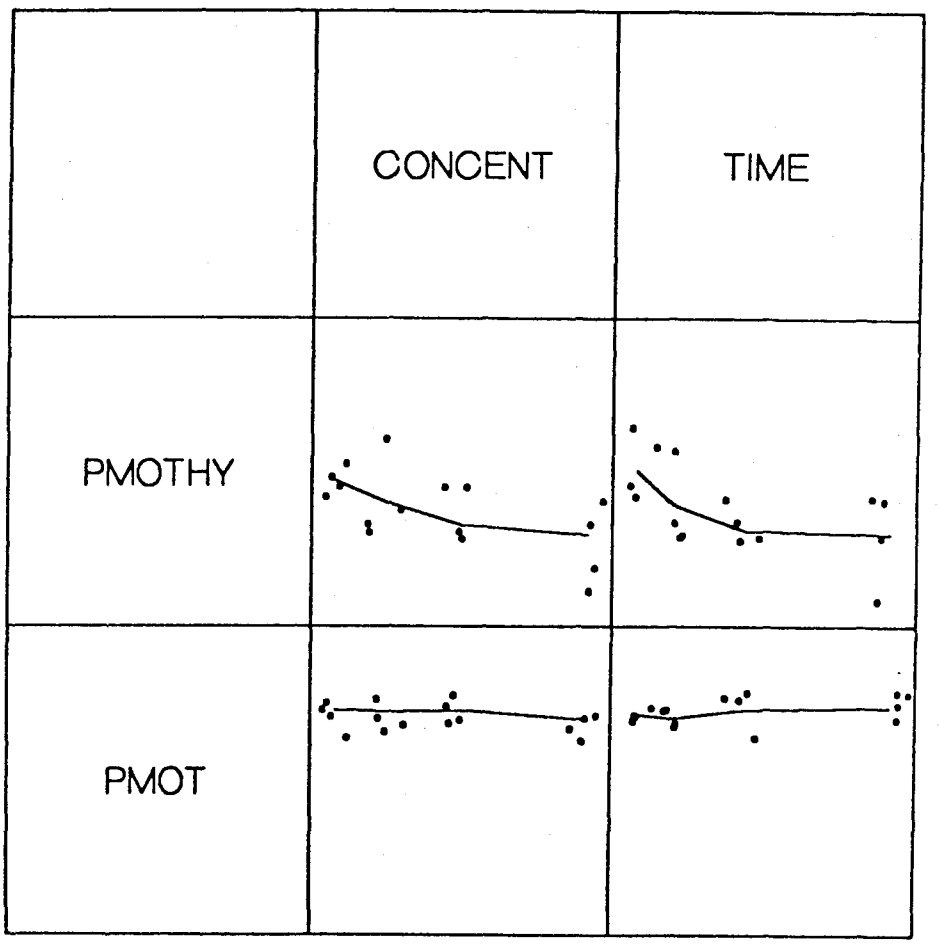

Figure 10. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 1.0 μM, 2.5 μM, and 5.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

c. Rabbit 793 (Cr)

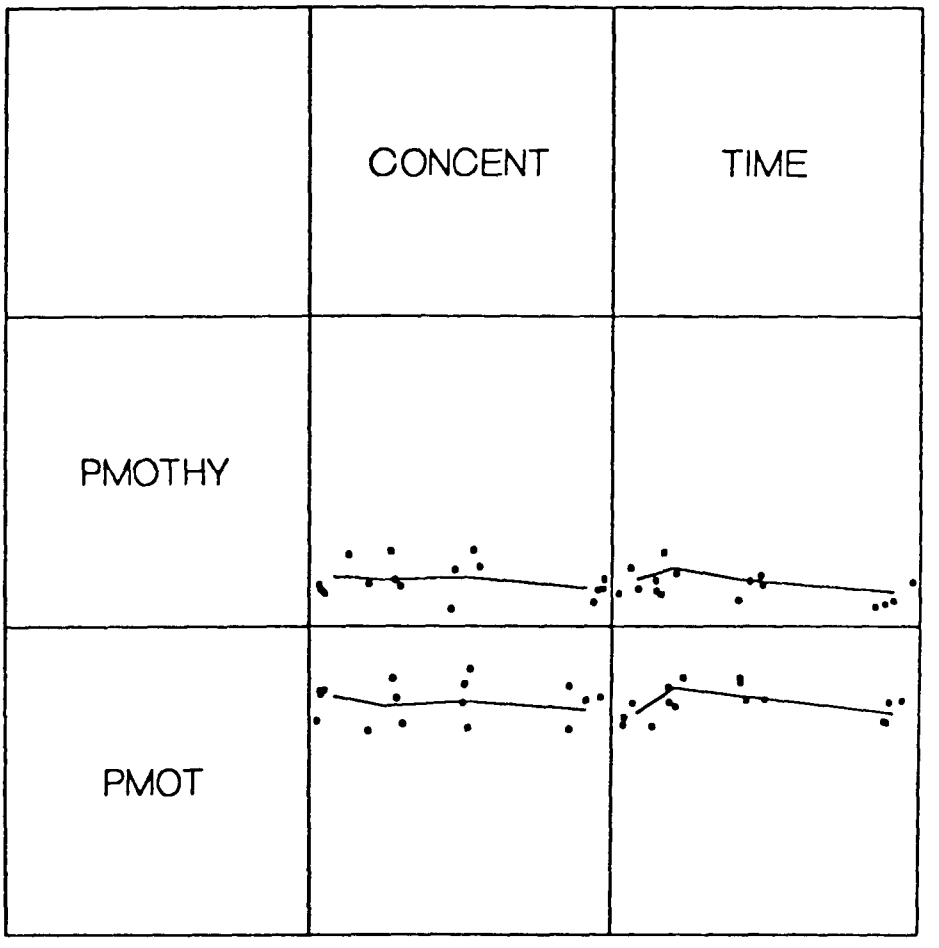

Figure 10. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 1.0 μM, 2.5 μM, and 5.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

d. Rabbit 967 (Cr)

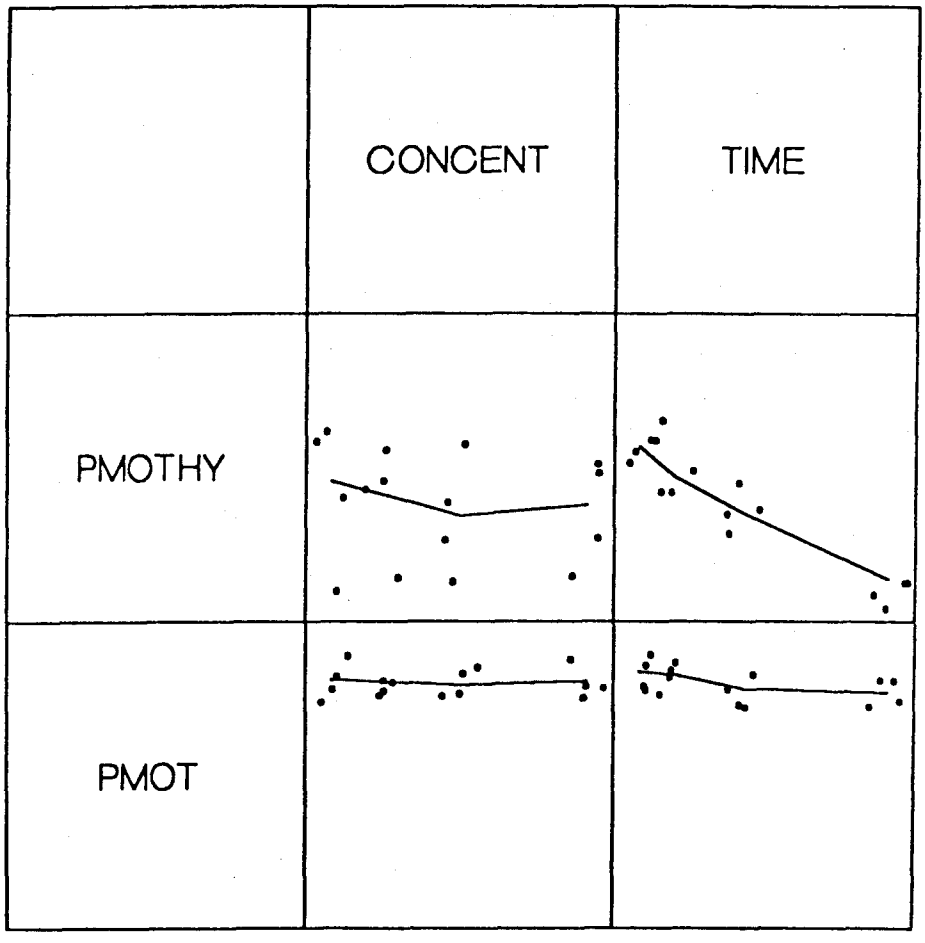

Figure 10. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 1.0 μM, 2.5 μM, and 5.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) (Continued)

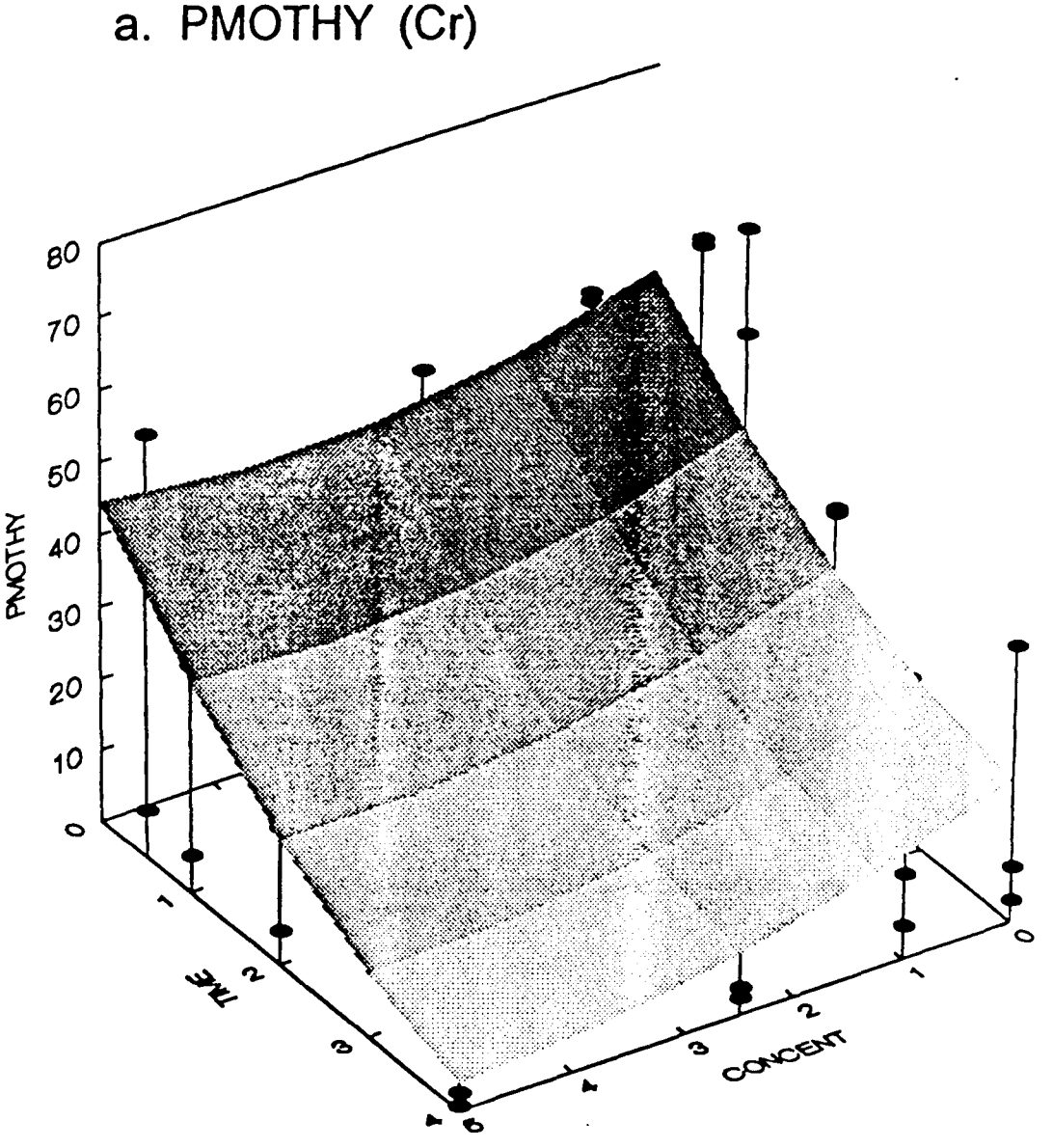

Figure 11. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Cr Concentration (μM) and Time (hr)

b. PMOT (Cr)

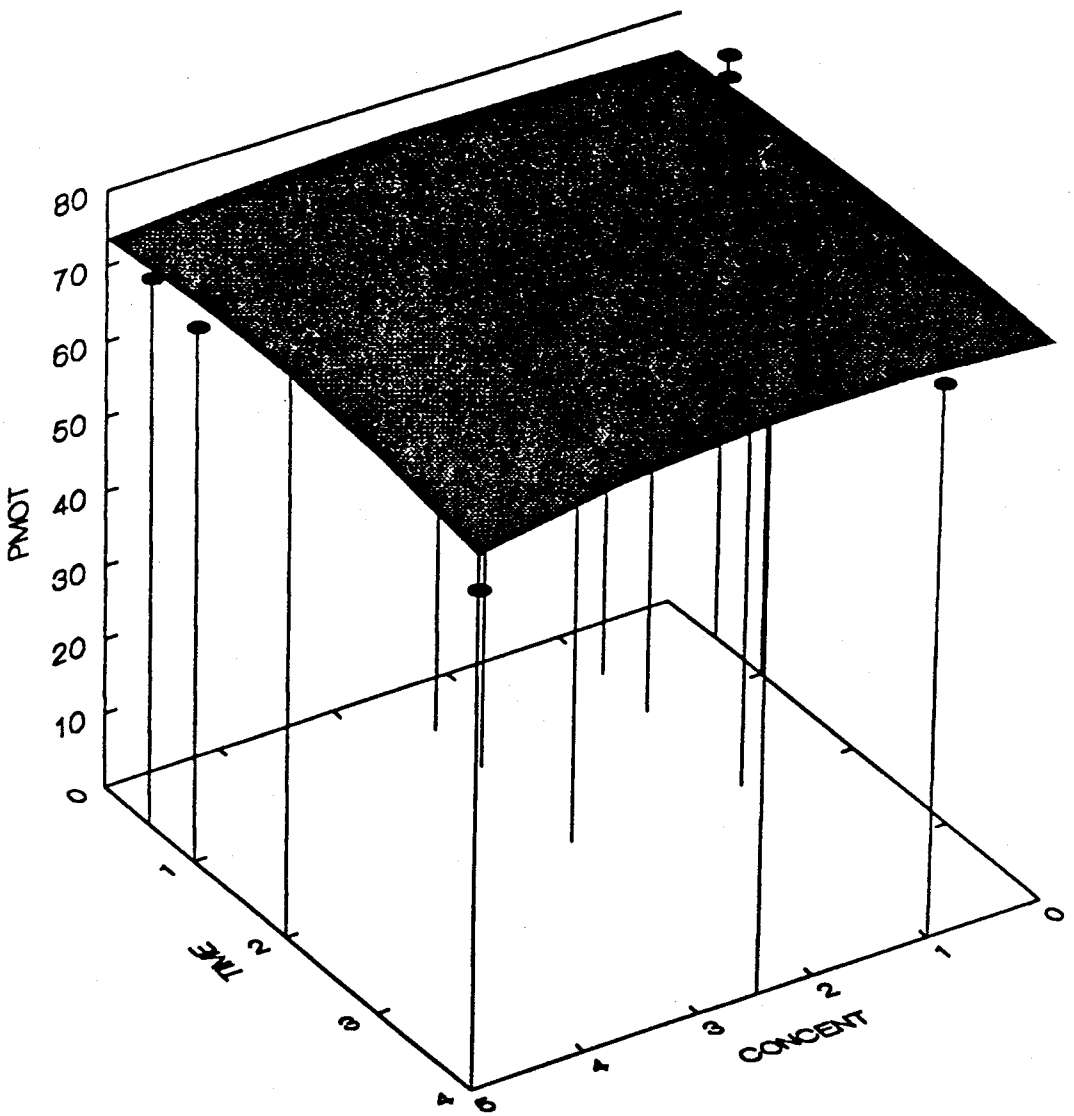

Figure 11. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Cr Concentration (μM) and Time (hr) (Continued)

With regard to nonhyperactivated motility, the response vector (AALH, LIN, VCL, and WOB) was not significantly influenced by changes in concentration or time (Figure 12).

3.6 Lead.

The data set for PMOTHY and PMOT consisted of 80 observations, 5 rabbits X 4 concentrations X 4 times. MANOVA showed a significant interaction between concentration and time. The AOV indicated the effect to be attributable to PMOTHY. The progression over increasing times for the response vector means PMOTHY, PMOT is for the control 22.3, 67.4; 18.5, 65.2; 8.5, 66.2; and 3.3, 60.1; for low concentration 21.3, 63.3; 9.5, 64.8; 6.4, 63.2; and 4.7, 55.2; for medium concentration 19.6, 64.4; 7.5, 62.6; 7.1, 53.8; and 3.5, 54.6; and for high concentration 9.9, 63.1; 5.6, 59.3; 4.3, 60.1; and 4.6, 55.5. The cause for the statistical significance is that the progression over time is not the same for each concentration. For the control, PMOTHY gradually decreases over time from 22.3 to 3.3; whereas, for the highest concentration, the progression is relatively flat, 9.9 to 4.6. The inference is that the PMOTHY was more quickly negatively affected with increased concentration.

The above results do not indicate the influence of concentration or time individually on the response vector. Generally, once a significant interaction is found, it is considered somewhat redundant to test the main effects, as they do influence the response in conjunction with other factors. Figure 13a-f and Figure 14a and b illustrate the associations among PMOTHY, PMOT, concentration, and time.

With regard to nonhyperactivated motility, the response vector (AALH, LIN, VCL, and WOB) was not significantly influenced by time, concentration, or their interaction (Figure 15).

3.7 Summary of Statistical Analyses.

For lead and cadmium, the percentage of motile cells mimicking hyperactivated motion was diminished with increased concentration or increased exposure (time X concentration). The percentage of motile cells and measures of the motility of the motile but nonhyperactivated cells were not (except for the VCL of cells in the presence of cadmium) significantly altered. For chromium, no statistically significant effect was observed for concentration or time on either response vector. Neither zinc nor mercury showed any change over concentration or time for the response vector describing nonhyperactivated motility. However, zinc and mercury showed a negative association between time and the percentage of motile cells that were hyperactivated. In addition, the percent motile decreased over time for mercury, and, on the strength of one point, over concentration for zinc.

 Text continues on page 55.

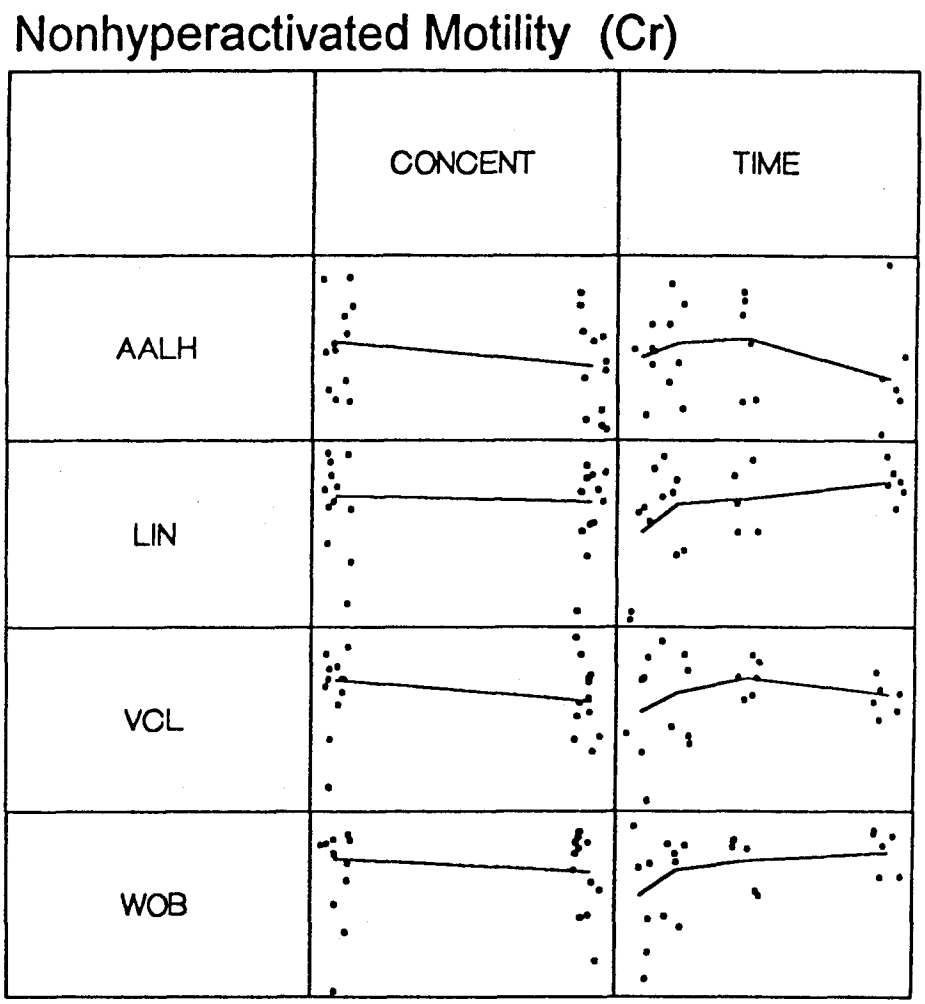

Figure 12. Scatterplot Matrix Depicting the Association Between Each of Cr Concentration (x-Axis Left to Right: 0 μM, and 5.0 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) and Nonhyperactivated Motility Represented by the Motility Parameters: AALH (Minimum 3.3 μm, Maximum 5.4 μm), LIN (Minimum 0.32, Maximum 0.85), VCL (Minimum 43 μm/s, Maximum 91 μm/s), and WOB (Minimum 0.46, Maximum 0.91)

a. All Rabbits (Pb)

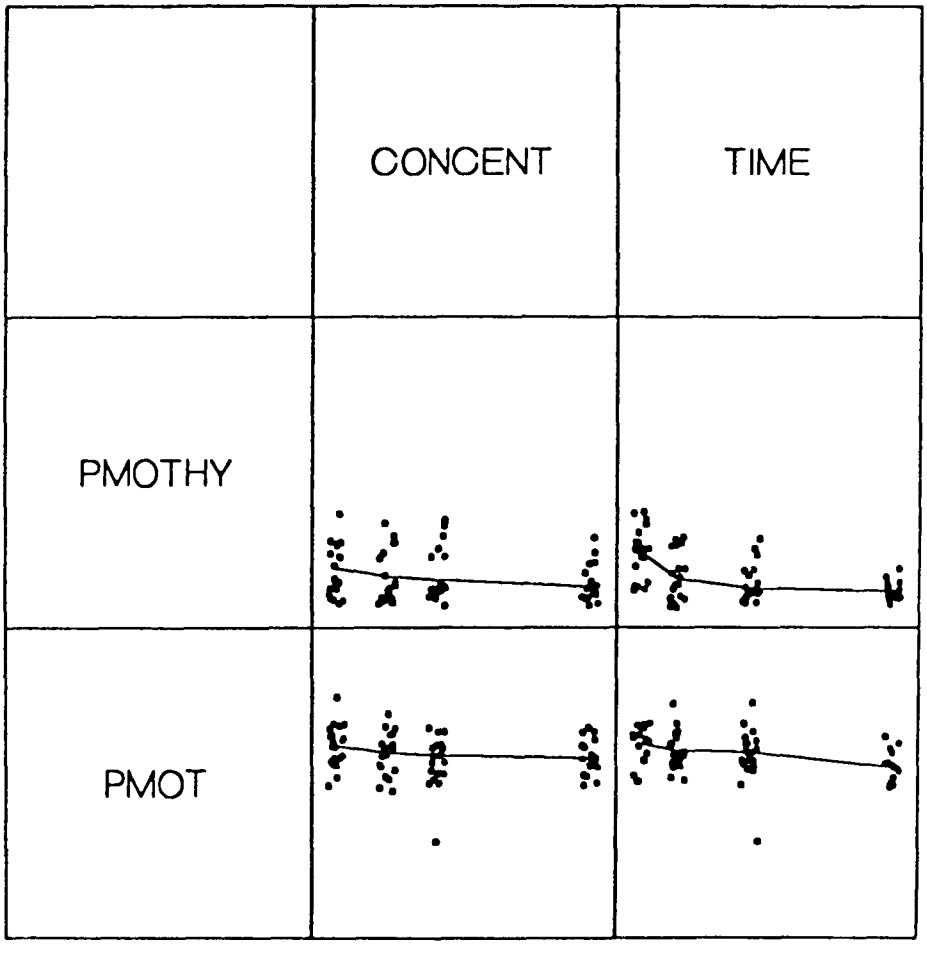

Figure 13. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 5 μM, 10 μM, and 25 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr). Two Rabbits were Used Twice but on Different Days

b. Rabbit 772a (Pb)

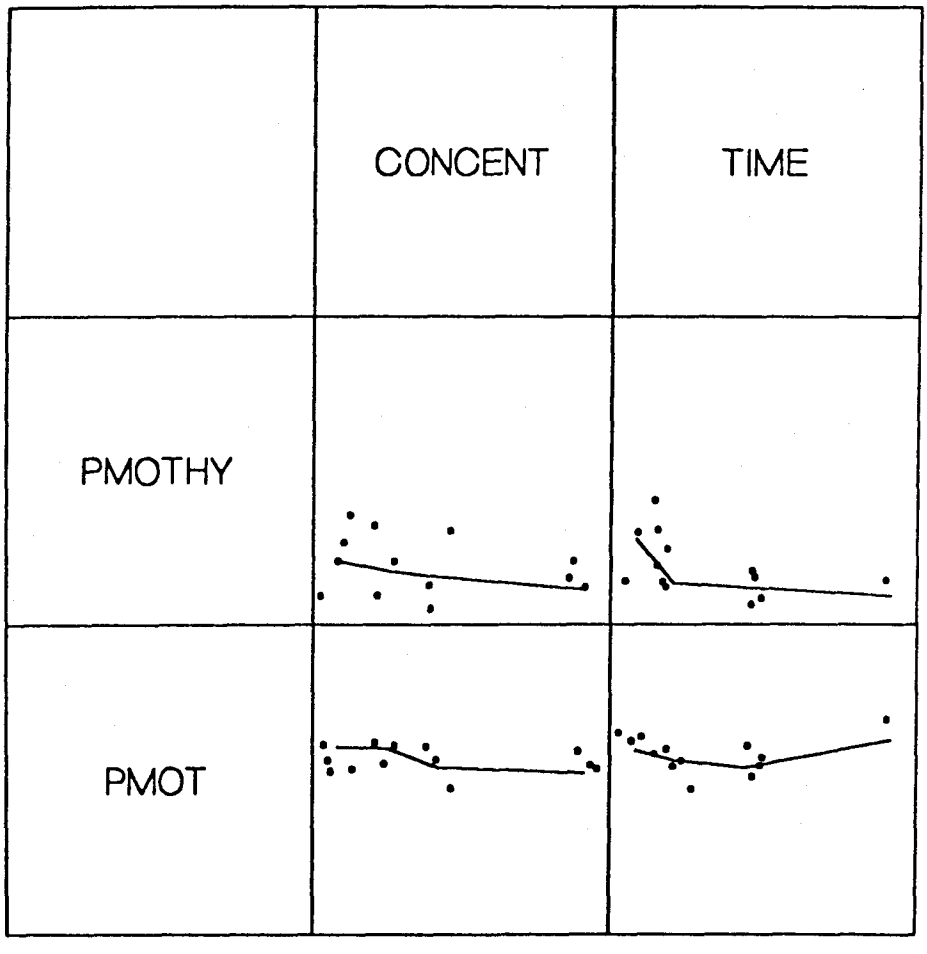

Figure 13. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 5 μM, 10 μM, and 25 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr). Two Rabbits were Used Twice but on Different Days (Continued)

c. Rabbit 854a (Pb)

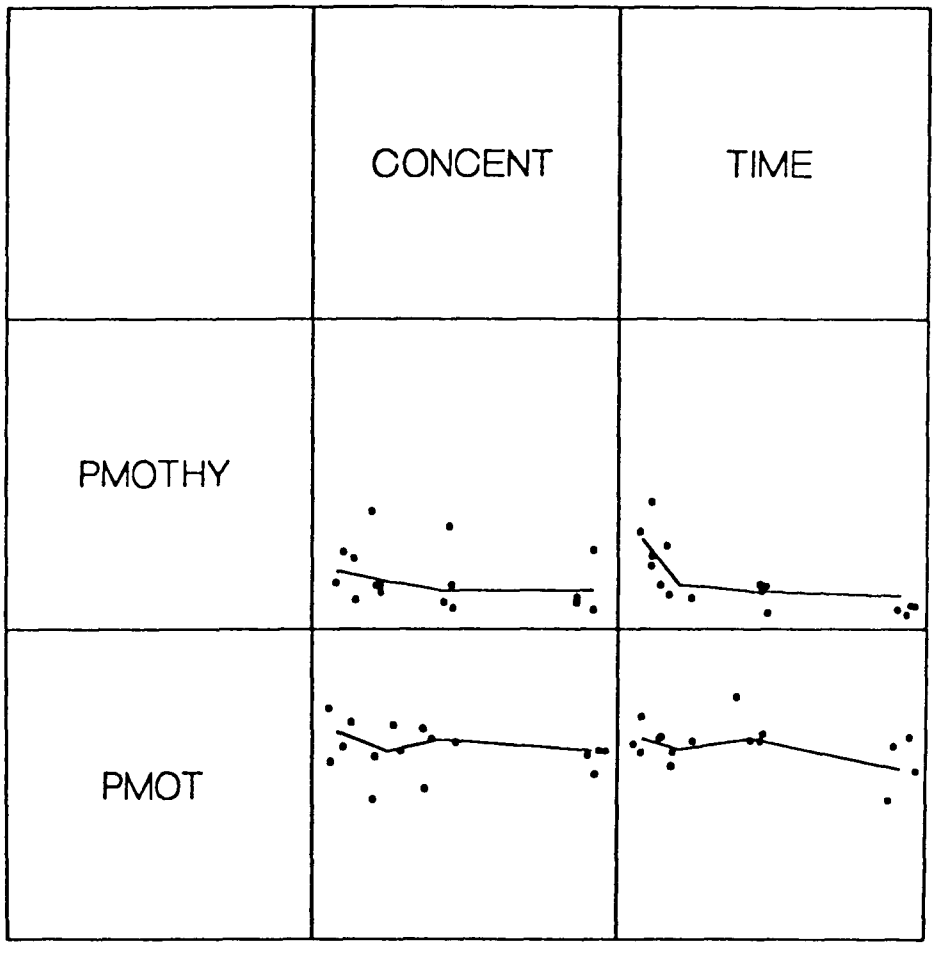

Figure 13. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 5 μM, 10 μM, and 25 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr). Two Rabbits were Used Twice but on Different Days (Continued)

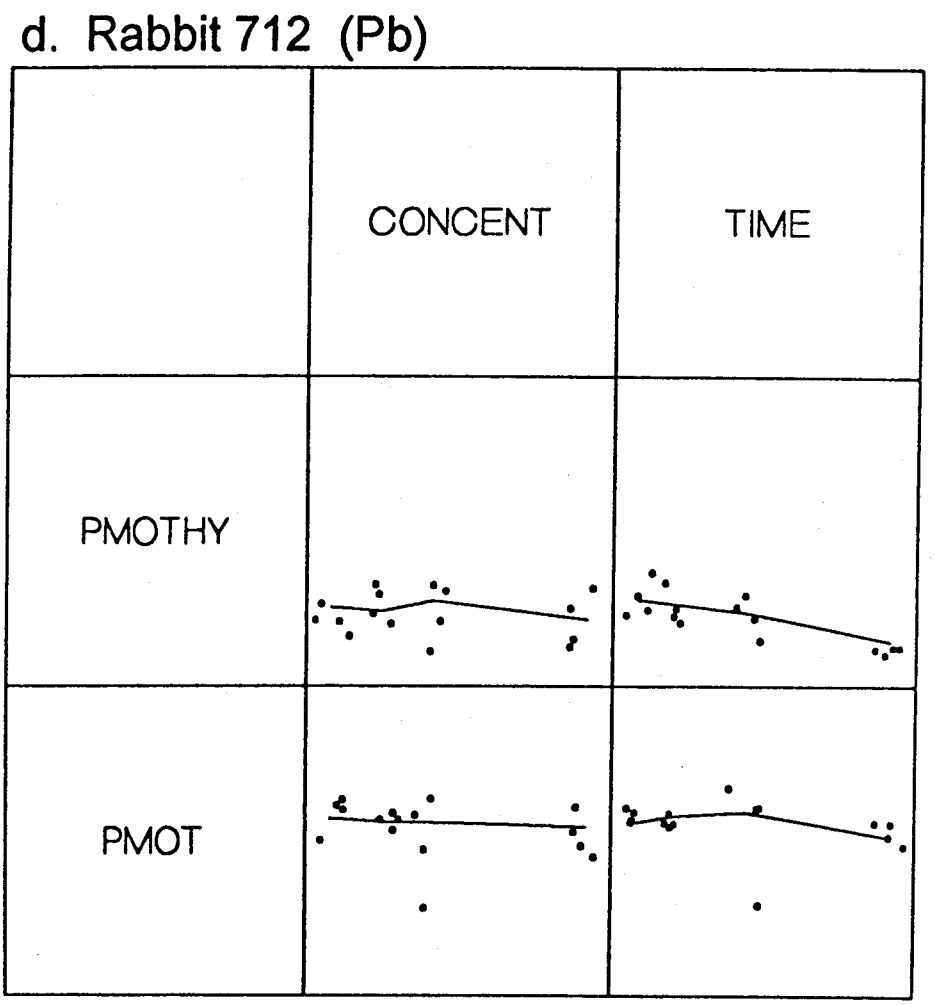

Figure 13. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 5 μM, 10 μM, and 25 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr). Two Rabbits were Used Twice but on Different Days (Continued)

e. Rabbit 772b (Pb)

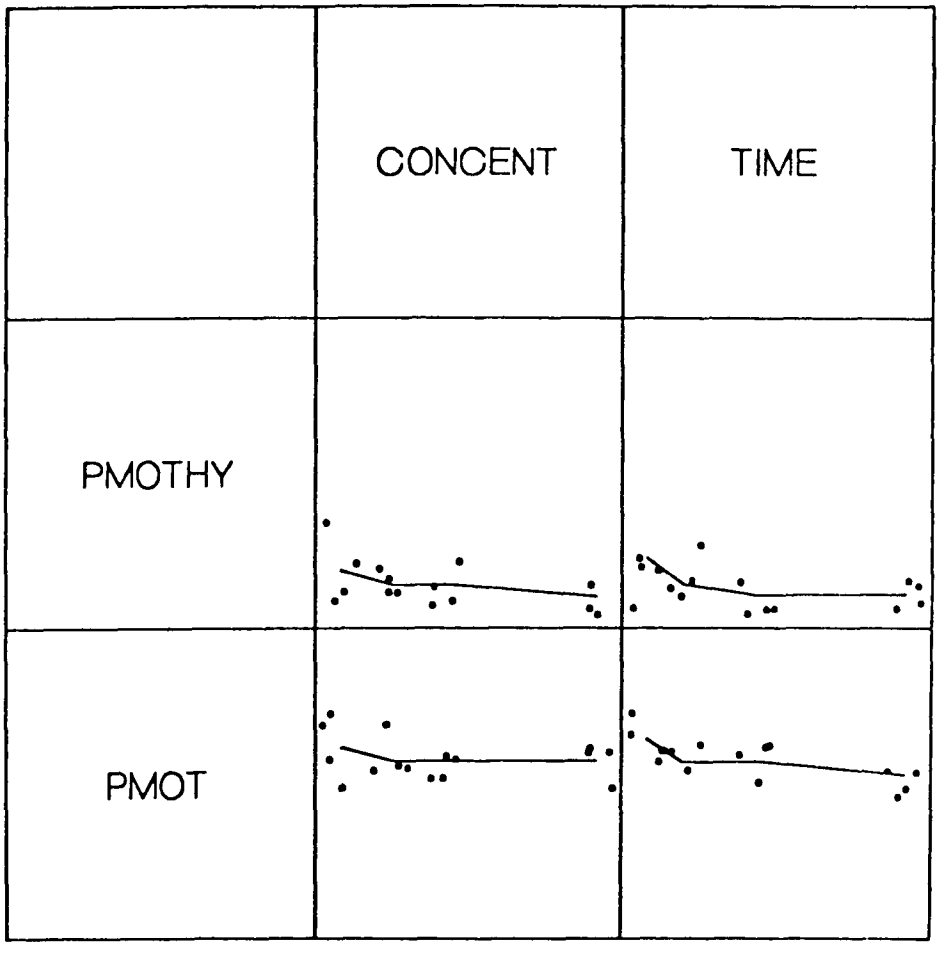

Figure 13. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 5 μM, 10 μM, and 25 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr). Two Rabbits were Used Twice but on Different Days (Continued)

f. Rabbit 854b (Pb)

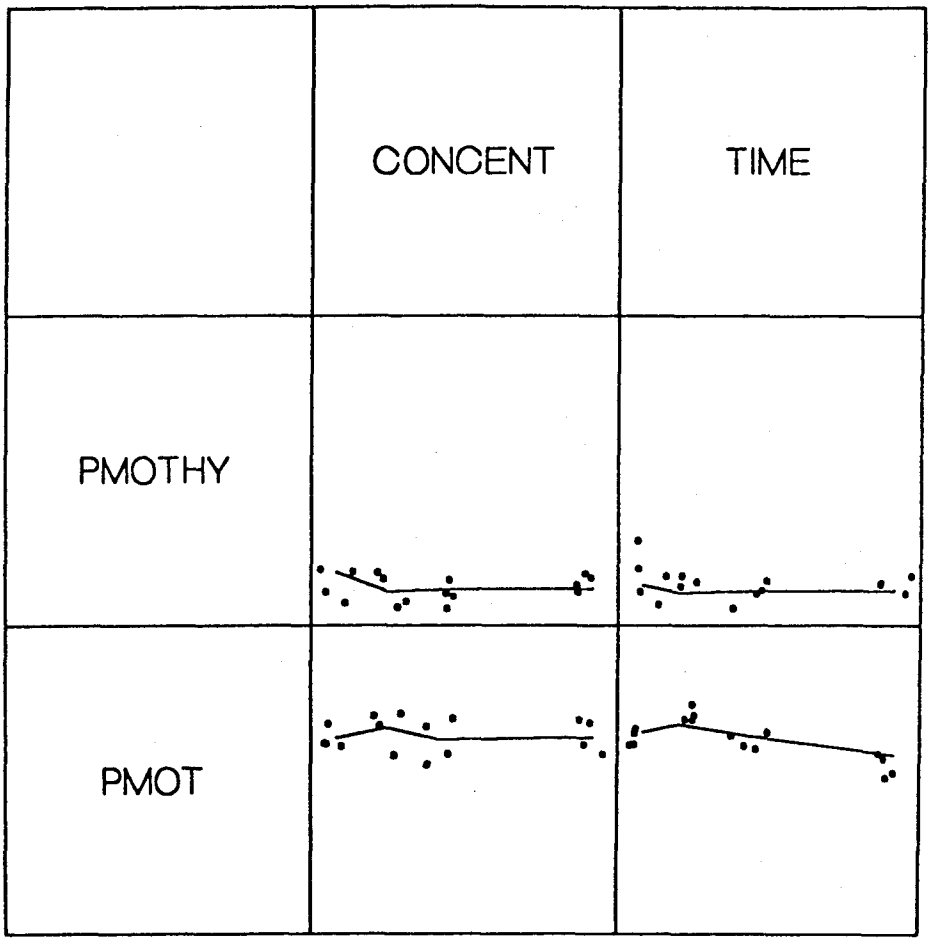

Figure 13. Scatterplot Matrices Showing the Association Between Each of PMOTHY and PMOT (y-Axes Extend from 0 to 100%) and Both Concentration (x-Axis Left to Right: 0 μM, 5 μM, 10 μM, and 25 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr). Two Rabbits were Used Twice but on Different Days (Continued)

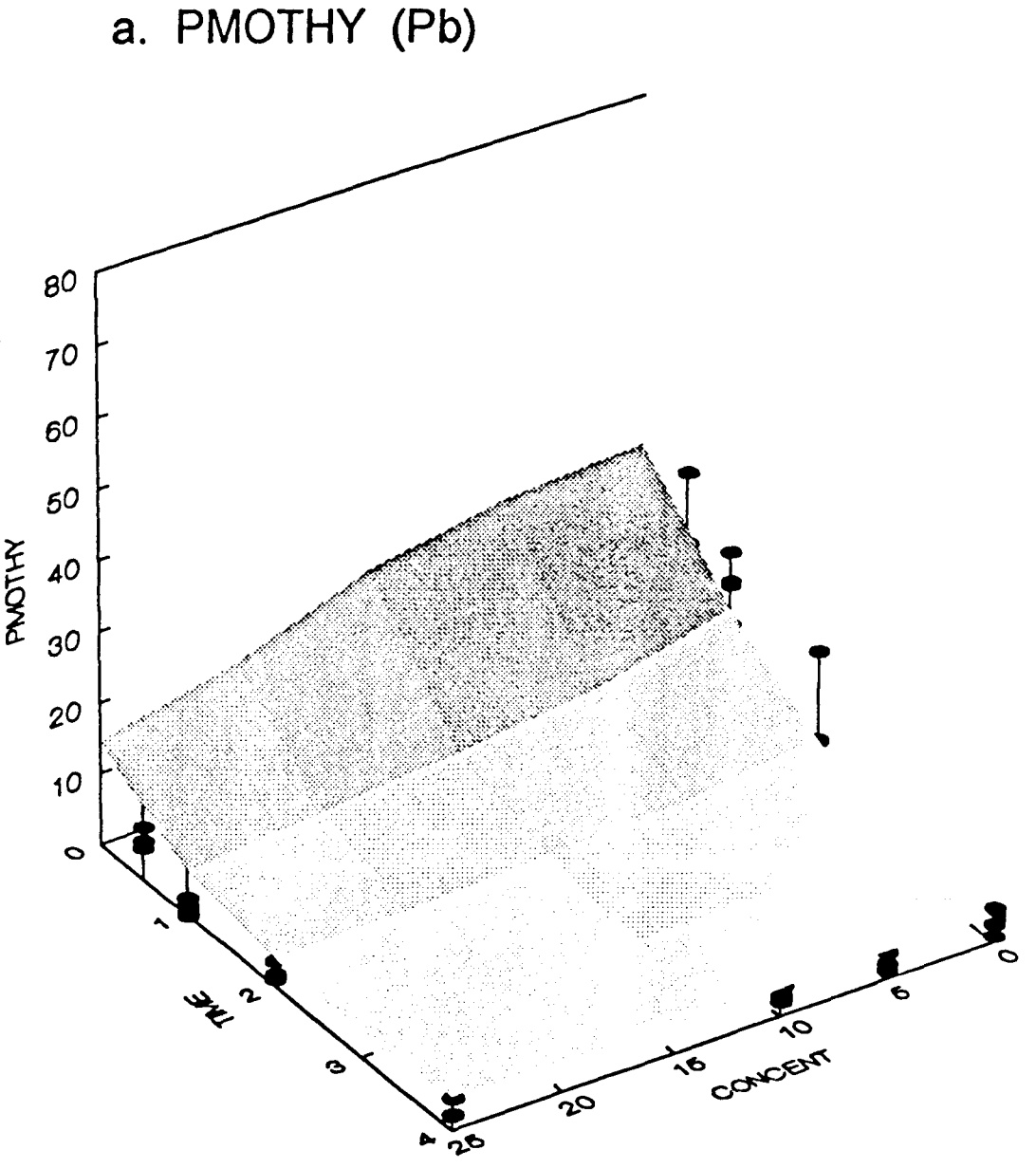

Figure 14. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Pb Concentration (μM) and Time (hr)

b. PMOT (Pb)

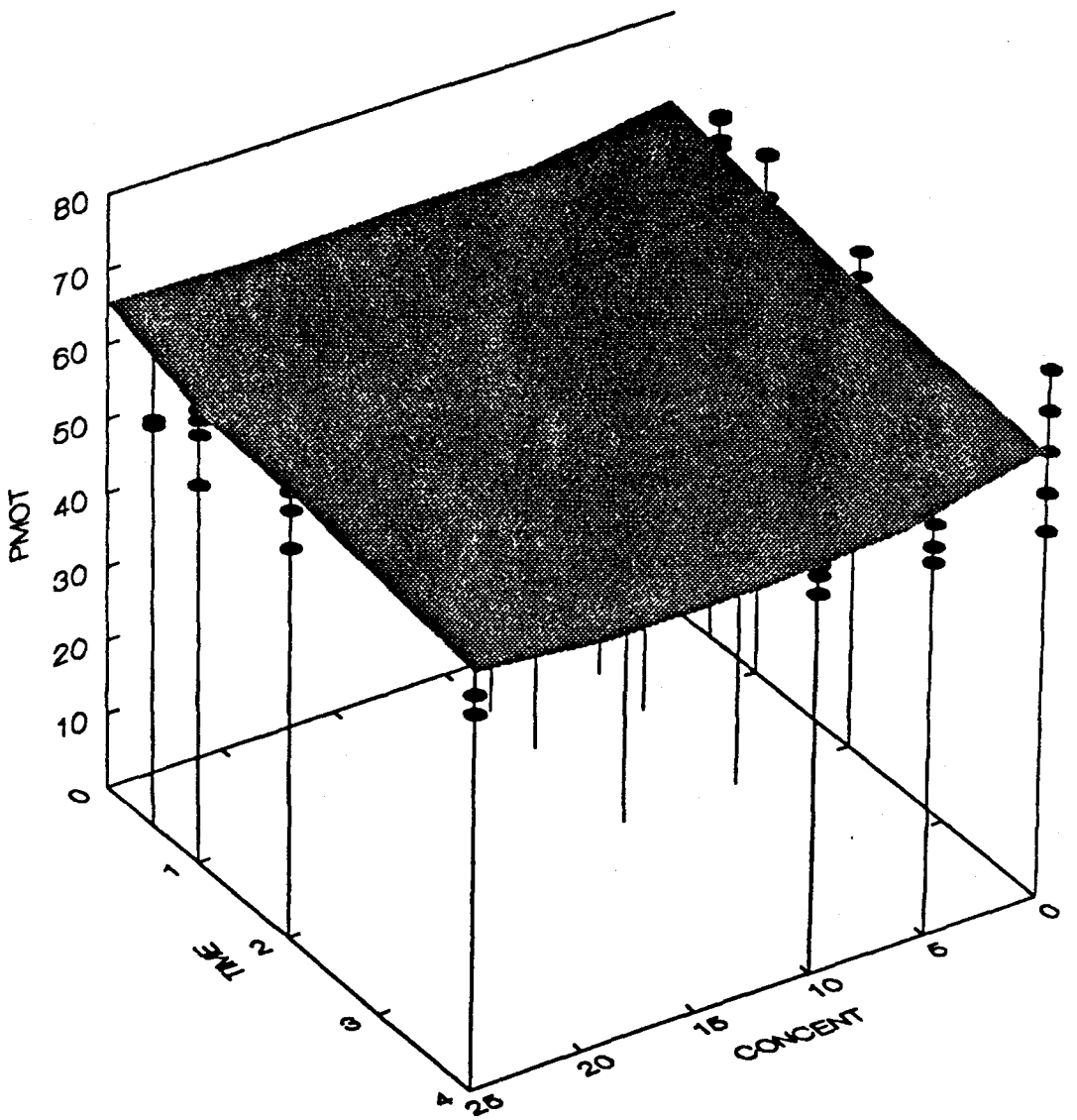

Figure 14. Three Dimensional Smoothed Scatterplots (Points, Not Obscured by the Surface, Shown) Illustrating the Combined Association of Pb Concentration (μM) and Time (hr) (Continued)

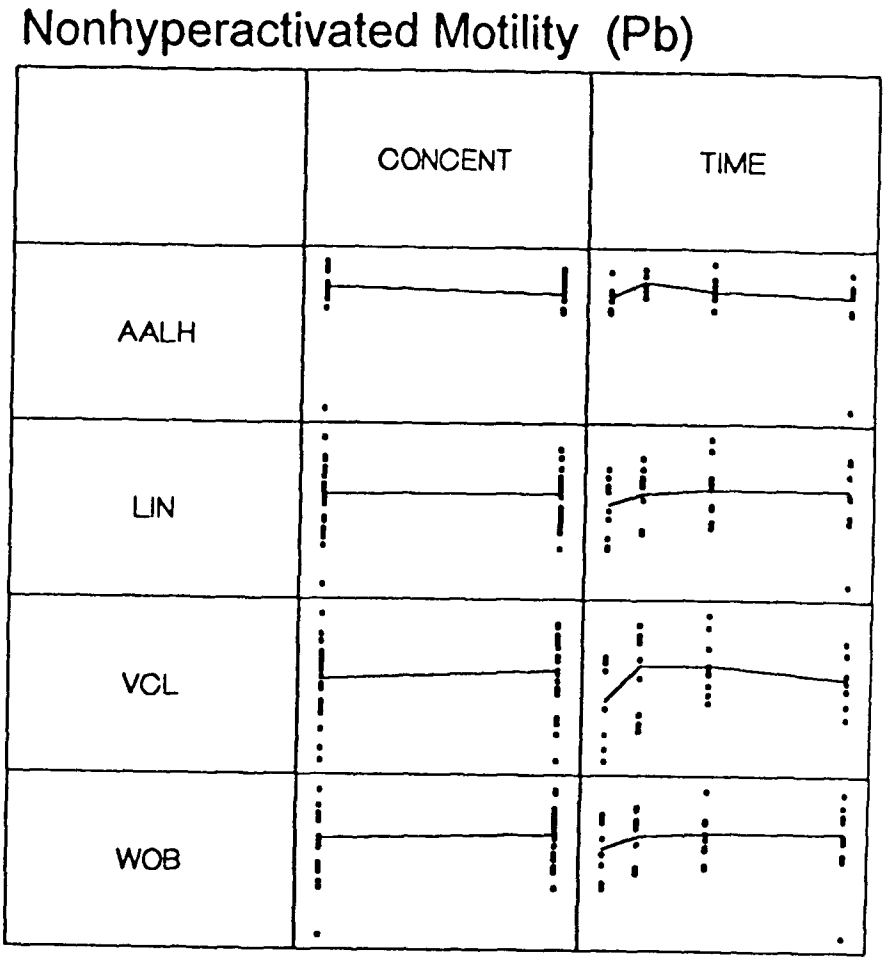

Figure 15. Scatterplot Matrix Depicting the Association Between Each of Pb Concentration (x-Axis Left to Right: 0 μM, and 25 μM) and Time (x-Axis Left to Right: 0.5 hr, 1 hr, 2 hr, and 4 hr) and Nonhyperactivated Motility Represented by the Motility Parameters: AALH (Minimum 0.4 μm, Maximum 5.2 μm), LIN (Minimum 0.36, Maximum 0.89), VCL (Minimum 51 μm/s, Maximum 111 μm/s), and WOB (Minimum 0.40, Maximum 0.95)

4. DISCUSSION

An appropriately conservative approach was adopted for the report of statistical findings. Ten separate multivariate analyses were conducted, two for each of the five metals. The change over concentration or time for any of the individual responses was pursued only after first observing a significance in the multivariate test. The multivariate tests are known to be less powerful than their univariate counterparts. In some instances (i.e., chromium), significant univariate tests were not reflected in the MANOVA findings. In that situation, it is most appropriate to only report the MANOVA results. Lead and cadmium are testicular poisons, and exposure to these metals have been associated with spermatogenesis and reproduction failure in men and in experimental animals.[14-18] The ability of lead and cadmium to inhibit hyperactivated motility, without a concomitant effect on nonhyperactivated motility, show that these two metals can have a second site of action as reproductive toxicants, namely inhibition of fertilization rather than impairment of spermatogenesis. Thus, exposure to the metals without consequential testicular dysfunction or alteration in sperm motility can result in fertilization failure. This may be an explanation for idiopathic infertility, and unexplained reproduction anomalies observed following exposure to the metals.[18,19]

The remaining three metals studied had no effect on development of hyperactivated motility or motions that mimicked hyperactivated motility. These metals also appear not to disturb fertilization. Zinc is essential for spermatogenesis and has not been implicated in fertilization error.[20,21] Exposure of rats and Macaca fasciularis monkeys to subneurotoxic doses of mercuric or methylmercuric chloride altered sperm production. Methylmercury produced a reduced litter size in exposed rats and mice.[22,23] However, the reduced litter size resulted from implantation impairment rather than fertilization failure.[24] Hexavalent chromium disrupted testicular function in rodents, but the effects on sperm quality in exposed men were equivocal and fertility of exposed men appear not to be compromised.[25-30]

The ability of the five metals to inhibit the development of hyperactivated motility by rabbit sperm in medium M parallels the fertility consequences of exposure to the metals. Monitoring the acquisition of hyperactivated motility may provide a guide to the potential of metals or other compounds to prevent fertilization.

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